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THE EFFECT OF LONG CONTINUED SUBCURATIVE DOSES OF PENICILLIN DURING THE INCUBATION PERIOD OF EXPERIMENTAL SYPHILIS*

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In the search for the most satisfactory and practical therapy for syphilis, the effects of penicillin on syphilitic infection have been studied in some detail, both in man and in rabbits. The effects of subtherapeutic amounts of penicillin have, however, received relatively little attention, although this aspect of the subject is of great practical interest, since most conditions for which penicillin is used usually require much smaller doses for adequate therapy than does syphilis. Of particular importance is the question of whether the use of penicillin in the treatment or prophylaxis of gonorrhea or some other infectious disease may modify the clinical course or mask the symptoms of an incipient syphilitic infection.

CLINICAL BACKGROUND

Most syphilologists believe as a result of their clinical observation and experience that the subsequent course of syphilis may be altered if subcurative amounts of penicillin are given during the incubation period. A number of reports have appeared (1, 2, 3, 4, 5) of examples of syphilitic infections in which the primary lesions appeared only after very long incubation periods, and in which the delay was attributed to the use of penicillin for the treatment of gonorrhea.

It has also been suggested that penicillin if given during the incubation period may alter the appearance of lesions or may even totally suppress the primary stage, but no clinical examples of this have been presented. Indeed, syphilis in man is so variable, both as to its incubation period, and in the location and the character of the initial lesions, that it is exceedingly difficult to define the limits of the normal clinical picture.

EXPERIMENTAL BACKGROUND

Alteration in the course of a syphilitic infection by small doses of penicillin during the incubation period can be more clearly and easily demonstrated in experimentally infected rabbits, where the conditions, particularly of exposure

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and re-exposure can be controlled. While this subject has not previously been systematically investigated, a number of pertinent observations have been made during the course of other studies.

Chesney and Kemp (6) demonstrated that the incubation period of experimental syphilis varied with the size of the infecting dose. Later Magnuson, Eagle, and Fleischman (7) extended these studies and, assuming that the increase of treponemes in the body was a steady logarithmic process, concluded that the generation time of *Treponema pallidum* under these circumstances was about 30 hours. Cumberland and Turner (8) approaching the problem by direct spirochete counts on infected rabbit testes, demonstrated that there was in fact a steady logarithmic increase in the number of treponemes during the incubation period, and from their data, derived a generation time of approximately 33 hours.

It is clear from the studies of Rich, Chesney, and Turner (9), that there are present during the incubation period, i.e., the period between inoculation and the development of a clinically perceptible lesion, specific histologic changes which are detectable a considerable time before the gross lesions appear. Eventually with the progressive increase in the number of treponemes at the local site, these changes give rise to a clinically detectable lesion. From the evidence in some of the foregoing work (7, 8) and from the present studies it appears that a fairly constant minimum concentration of treponemes in the order of 10^7 organisms is required for a lesion to be clinically perceptible.

It can be surmised on theoretical grounds, as well as on the basis of experience with other organisms that a specific subcurative dose of penicillin will kill a fairly constant proportion of the treponemes present. This is supported by the results of two prior studies (3, 10).

Magnuson and Eagle (3) found that when penicillin was given to rabbits during the incubation period of experimental syphilitic infection, more rabbits were cured when treatment was begun early. The remaining animals, however, for which the dosage was subcurative, all responded with the same prolongation of the incubation period whether the treatment was begun one hour after, or 3, 7, 10, or 14 days after inoculation (Table 1). Apparently the same proportion of the spirochetes had been destroyed by the same dosage irrespective of the time of treatment.

Rake, Dunham and Donovick (10) also treated rabbits during the incubation period, but gave various doses of penicillin at a fixed interval after intracutaneous inoculation. They found that the length of the incubation period as well as the proportion of animals cured varied directly with the dosage.

Symptomless infection occurred rarely in these and similar experiments, and penicillin may not have been the immediate cause. For example, Eagle, Magnuson, and Fleischman (11) transferred the lymph nodes of 102 rabbits

which had failed to develop local lesions after intracutaneous or testicular inoculation and penicillin treatment. Only one of these animals was found to be infected, and since this animal had been inoculated intratesticularly it is conceivable that a slight reaction may have been undetected. Rake et al (10), found no infected rabbits among 101 which were symptom-free after intracutaneous inoculation and varying amounts of penicillin treatment. Three of 54 which failed to develop lesions after inoculation by scarification of the skin followed by penicillin were infected, but one of the 30 untreated controls also developed a symptomless infection when inoculated by this route.

In the present investigation the problem of the possible changes which can be induced in a syphilitic infection by the administration of a small amount of

TABLE 1

Prolongation of the Incubation Period Produced by Subcurative Doses of Penicilin (Data Rearranged from Magnuson and Eagle (3))

AMOUNT OF PENICILLIN	TIME OF TREATMENT	EXPERIMENT A			EXPERIMENT B		
		No. of rabbits	Mean incubation (days)	Prolongation (days)	No. of rabbits	Mean incubation (days)	Prolongation (days)
None	—	5	16	—	3	10	—
400 U (q 3 hr × 5)	1 hr.	5	28	12	3	35	25
400 U (q 3 hr × 5)	3 days	2	32	16	5	31	21
400 U (q 3 hr × 5)	7 days	2	39	23	6	37	27
400 U (q 3 hr × 5)	10 days	3	37	21	4	32	22
400 U (q 3 hr × 5)	14 days	4	28	12	2	35	25
Total Treated		16	31	15	20	34	24

penicillin during the incubation period, has been re-examined. In the preliminary stages of the investigation the course of syphilis in rabbits was studied after single small doses of penicillin. This was followed by a study of the effects of multiple small doses, given in schedules which were designed to maintain subclinical infection for long periods, i.e., up to 20 weeks.

EXPERIMENTAL METHODS

Adult male rabbits of mixed breeds weighing 3 to 4 kilo were used. The animal room was maintained at a temperature of 68°F or somewhat lower. Rabbits were fed "Purina" compressed food pellets. Water and salt were made freely available. Blood was ordinarily withdrawn in 10 cc amounts from the ear veins by sterile syringe and needle.

The Nichols strain of *T. pallidum* was used for all inoculations. Suspensions and dilutions were prepared with 10 per cent normal rabbit serum in 0.85 per cent saline and the organisms were enumerated in the manner previously described (8).

Penicillin G Sodium (Winthrop Stearns, Lot #4508C) was used in the preliminary experiments and Penicillin G Potassium (Parke-Davis and Co., Lot #016331E) in the long term experiment. Dilutions with saline were made directly in the vials immediately before

use, on the basis that each vial contained 60 mgm of penicillin. Repeated assays against a standard strain of staphylococcus showed that, per vial, the two brands were of equal potency and that each vial contained approximately the amount stated. Penicillin dosage was always calculated for the weight of the animal on the day of injection. The solution was given intramuscularly in the lateral muscles of the right thigh.

Sera were examined for Wassermann reagin by the Eagle Flocculation and V.D.R.L. tests. Treponemal immobilizing antibody was tested by the method of Nelson and Mayer (12), or in the case of anti-complementary sera, by a modification of this method devised by Barron and Mayer (13).

EXPERIMENTAL RESULTS

Effect of Single Small Doses of Penicillin

The purpose of the preliminary experiments was to examine the influence of a single small dose of penicillin on the progress of a syphilitic infection and particularly the incubation period. Data from three separate experiments are presented in Chart 1.

In each experiment rabbits were inoculated with a single suspension of *T. pallidum* so as to receive 500 organisms intracutaneously at each of four sites on the clipped surface of the back. The incubation periods in relation to the amounts of penicillin given in single doses are shown in Chart 1. Ordinarily lesions developed simultaneously at all four inoculation sites in each animal and rapidly enlarged during the next few days. Infection in each animal was confirmed by demonstration of treponemes with the darkfield microscope.

The incubation period of the untreated animals was quite uniform in each experiment, and strikingly similar in the three experiments, although they were performed on different days with different inocula. This is in accord with the experience with similarly inoculated animals in this laboratory over a period of years.

The effect of a small dose of penicillin given during the incubation period was definite and constant in the three experiments. Each group of the treated rabbits which received at least 0.1 mgm per kilo of penicillin showed a significantly longer incubation period than the untreated controls. Furthermore, as is shown in Chart 1, the larger the dose of penicillin given, the greater in general was the prolongation of the incubation period. Serological examinations two or three times a week during these extended incubation periods revealed that the production of Wassermann reagin was postponed, and that this did not appear in any animal until after lesions had developed.

In the third preliminary experiment shown in Chart 1 two groups of rabbits were also given a second dose of penicillin one week after the first injection. It was apparent, that with two doses, the prolongation in each case was approximately double that obtained by single doses. This prolongation effect produced by repeated doses of penicillin at appropriate intervals was examined in more detail in the following experiment.

Effect of Small Doses of Penicillin at Weekly Intervals

Forty-six rabbits were inoculated intracutaneously on the back with 500 treponemes at each of 4 sites. 42 of these were then assigned to a treatment schedule as shown in Table 2, so that 4 animals were untreated, 4 received 2 treatments, 4 animals 4 treatments and so forth up to a maximum of 20 injections. The first dose was given on the 12th day after inoculation and later doses at weekly intervals thereafter. Two of each group of four treated animals

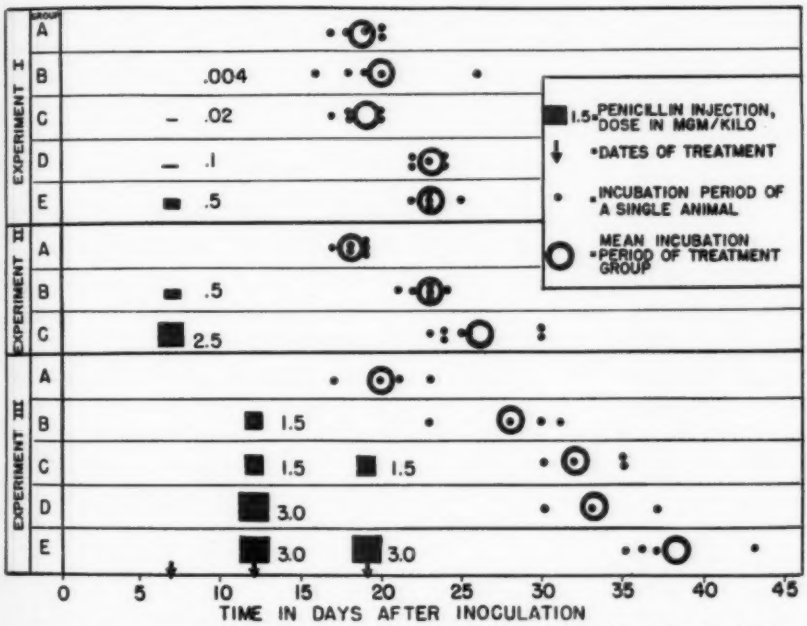

Chart 1. Prolongation of the Incubation Period of Syphilis in Rabbits After Small Doses of Penicillin

received 2 mgm crystalline penicillin G per kilo and the other two 4 mgm per kilo. The untreated control rabbits developed typical lesions after 15, 17 and 19 days. (A fourth animal died.)

The treated animals can be divided into three main categories according to the eventual outcome of their syphilitic infection (Table 2). Seventeen animals were symptom free at the end of their treatment schedule, and, on the basis of further observations including lymph node transfers, were classed as cured. Nineteen animals were symptom free at the end of treatment but were still infected and subsequently developed lesions; and 4 animals presented progressive lesions during the treatment. It is noteworthy that no animal

that was negative at the end of treatment and remained symptom free during the next two months was subsequently proven to be infected. Two rabbits died before the study was completed.

TABLE 2

Outcome of Syphilitic Infection in 42 Rabbits After Repeated Doses of Penicillin at Weekly Intervals (Numbers refer to the days after inoculation on which lesions appeared)

NUMBER OF TREATMENTS	PENICILLIN 2 MGM/K				PENICILLIN 4 MGM/K			
	Rabbit No.	Result			Rabbit No.	Result		
		Cure	Partial Suppression	Complete Suppression		Cure	Partial Suppression	Complete Suppression
2	30-77			34	30-98			31
	30-94			31	31-15			30
4	30-80			48	31-01	X		
	30-91			43	31-12			49
6	30-83			66	31-04	X		
	30-88	X			31-09			106
8	30-85		24		31-06			85
	30-86			87	31-07	X		
10	30-82			98	31-03	X		
	30-89		41		31-10	X		
12	30-79		66		31-00			101
	30-92		41		31-13	X		
14	30-76		(Died)		30-97			121
	30-95	X			31-16	X		
16	30-78			140	30-99	X		
	30-90	X			31-11	X		
18	30-81			146	31-02	X		
	30-93			144	31-14	X		
20	30-84			167	31-05		(Died)	
	30-87	X			31-08	X		
	30-96			156	31-17	X		

Animals Cured by Repeated Small Doses—Clinical Course

Of the 21 animals that received the smaller penicillin dose 4 were cured during the treatment, while 13 of 21 receiving the larger dose were cured (Table 2).

Of these 17 cured animals only three showed any trace of a lesion *during* the treatment period. One of these had a slight reaction at the 4 sites of inoc-

ulation after the second dose; a second had a slight reaction after both the first and second doses; and the third developed a tiny papule after the third dose which persisted until the sixth dose, at which time two more tiny lesions appeared for a few days, and then all disappeared.

Only four of the 17 cured animals showed any trace of a lesion *after* the treatment period. In each of these four, about two weeks after the last penicillin dose a very indefinite reaction was noted in the neighborhood of one or more of the inoculation sites. This appeared as an ill-demarcated zone of erythema with increased growth of hair and a suggestion of a central depression. Definite papules were never present and repeated darkfield examinations were always negative. The nature of these lesions remains obscure.

Serological tests for Wassermann reagin in these 17 cured animals were consistently negative. Treponemal immobilization tests on sera taken after the 7th and 20th weeks were negative in all 17 animals.

Animals Cured by Repeated Small Doses—Lymph node infectivity

Between the 60th and 90th day after the last treatment in each of the 17 cured animals the popliteal lymph nodes were removed, emulsified in 10 per cent normal rabbit serum in saline, and injected into one testis of each of two healthy normal rabbits. None of these 34 passage animals developed an orchitis or other evidence of syphilitic infection during a 90 day observation period.

As controls the popliteal lymph nodes from each of 8 rabbits in this same experiment which previously had had darkfield positive lesions and therefore were known to be infected, were similarly transferred to two normal rabbits. Fifteen of these 16 passage animals developed a typical specific orchitis within 6 weeks.

Animals Cured by Repeated Small Doses—Challenge inoculation

Thirteen survivors of the cured group were reinoculated with 500 treponemes intracutaneously at 3 new sites on the back. All 13 developed lesions comparable in appearance to those seen in normal rabbits inoculated at the same time. One animal was remarkable in that lesions developed after an exceptionally short incubation period of 10 days compared with a mean period of 22 days for the controls. In this animal transient lesions had been present for almost a month.

As additional controls, 8 animals from this same experiment which were known to be infected, were challenged in the same manner. These animals remained completely free from reaction at the inoculation sites.

Animals with progressive lesions during repeated small doses

Four rabbits developed persistent lesions while they were receiving the smaller dose of penicillin. In two, papules appeared at all four inoculation

sites; and in two a single lesion developed first and others appeared later. As treatment continued the papules remained indolent, but at times would very slowly become larger or smaller with all the lesions in the same rabbit tending to follow the same course. In each animal treponemes were found by darkfield examination, but only after long search and repeated attempts. In none was there any tendency to ulceration during the period of treatment. Throughout this stage serological tests for Wassermann reagin and treponemal immobilizing antibody remained negative. At the time of the last treatment the 4 rabbits showed respectively 4 large lesions, 4 flat and inconspicuous lesions, 4 barely perceptible papules, and 2 fair-sized lesions.

About 12 days after the last dose of penicilin each of these 4 rabbits began to react in a more normal manner. The lesions rapidly increased in size and contained numerous spirochetes. At about the beginning of the fourth week after the completion of treatment ulceration appeared and the disease continued to proceed through its usual evolution.

Animals with Symptoms Suppressed by Repeated Doses of Penicillin

Nineteen animals were free from symptoms at the end of the treatment schedule, but later developed syphilitic lesions (Table 3).

Of the 19, 11 were completely symptomless throughout treatment, 4 were essentially so except that once or twice a suspicious reaction was noted at one or more of the inoculation sites; and 4 had transient small but definite papules which remained for 2 to 47 days during the treatment period.

Suppression by Penicillin—Incubation period of local lesions

Eighteen of the 19 animals in this group developed lesions at the inoculation sites. The total time from inoculation ranged from 30 days up to a maximum of 167 days in an animal which received 20 doses of penicillin.

More interesting is a comparison of the incubation period in relation to the termination of treatment, and the theoretical incubation period which might be expected under these circumstances (Chart 2).

It is apparent that the observed incubation periods, which are represented by solid spots, lie roughly along the straight diagonal line which connects the theoretical incubation periods. As explained later, the theoretical period is calculated by adding to the incubation period of the untreated controls, 7 days for each weekly penicillin treatment. In this experiment it can be computed by adding 12 days to the day of last treatment.

Suppression by Penicillin—Pattern of local lesions

Most animals developed lesions at each of the four inoculated sites, but 5 of the 18 animals that developed local lesions had a defective pattern in that

one, two, or three of the originally inoculated sites remained completely negative (Table 3). In addition, one infected animal developed no lesions at the inoculated sites but only generalized lesions which appeared somewhat later.

TABLE 3

Time of Appearance of Lesions in Animals which at the End of Treatment were without Lesions but Still Infected. (Numbers refer to days after inoculation)

RABBIT NO.	TREATMENT		INCUBATION PERIOD		LOCAL LESIONS		GENERALIZED LESIONS	
	Dosage mgm/k	Lesions During	Theoretical	Observed	No.	Character	Location	Date
30-77	2 × 2 wks	0	31	34	4	coalescent papules	0	—
30-94	2 × 2 wks	0	31	31	4	annular	skin	73
30-80	2 × 4 wks	0	45	48	4	usual	testis	104
30-91	2 × 4 wks	0	45	43	4	usual	skin	82
30-83	2 × 6 wks	0–?	59	66	4	multiple coalescent papules	testis	124
30-86	2 × 8 wks	0–?	73	87	1	usual	testis ulna	117 127
30-82	2 × 10 wks	0–?	87	98	4	one double	skin	137
30-78	2 × 16 wks	26–73	129	140	3	exceedingly bizarre	skin	169
30-81	2 × 18 wks	82–101	144	146	4	very atypical	0	—
30-93	2 × 18 wks	34–36	144	144	3	definitely bizarre	skin	172
30-84	2 × 20 wks	0	158	167	4	quite bizarre	0	—
30-96	2 × 20 wks	98–137	158	156	4	bizarre	0	—
30-98	4 × 2 wks	0	31	31	4	usual	0	—
31-15	4 × 2 wks	0	31	30	4	bizarre annular	skin	80
31-12	4 × 4 wks	0–?	45	49	4	multiple coalescent	0	—
31-09	4 × 6 wks	0	59	—	0	—	skin	106
31-06	4 × 8 wks	0	73	85	2	usual	skin	109
31-00	4 × 12 wks	0	101	101	4	usual	skin	143
30-97	4 × 14 wks	0	115	121	3	one double	0	—

Suppression by Penicillin—Atypical lesions

As indicated in Table 3 the lesions themselves varied from typical papules which went through a quite usual evolution, to lesions which began as double or multiple papules that coalesced and then resumed a usual course; and finally lesions which began as multiple papules, progressed very irregularly, and remained as unequal groups of small papules quite unlike any lesions ordinarily observed.

Suppression by Penicillin—Generalized lesions

Disseminated or generalized lesions were seen in 12 of the 19 infected animals. Nine of these animals had skin papules, while two had metastatic orchitis, and one orchitis along with a lesion of the ulna. The skin papules appeared from 29 to 49 days after the theoretical incubation period. (Table 3; Chart 2). Testicular lesions, which are harder to detect at an early stage, were noted in three rabbits on the 44, 59 and 65th day after the theoretical incubation period.

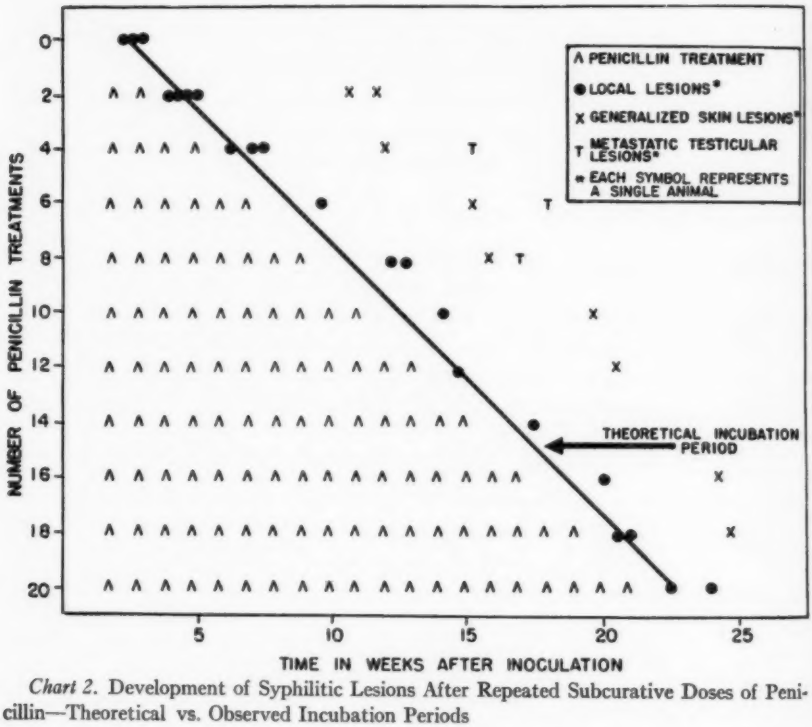

Chart 2. Development of Syphilitic Lesions After Repeated Subcurative Doses of Penicillin—Theoretical vs. Observed Incubation Periods

Suppression by Penicillin—Generalized without primary lesions

One rabbit (31–09), which failed to develop lesions at the inoculation sites, is of special interest, because it was nevertheless infected and later developed generalized lesions. Following the termination of treatment only a very faint questionable reaction was noted at the points of inoculation, and nothing further developed; yet, 59 days after the last penicillin dose or 47 days beyond the theoretical incubation period a number of rather large, but otherwise typical generalized skin papules appeared.

Suppression by Penicillin—Serology

All animals were bled at monthly intervals. The sera of all 14 animals in which local lesions were completely suppressed during the period of penicillin treatment, gave negative standard serological tests and treponemal immobilizing reactions throughout the incubation period. After symptoms were present Wassermann reagin appeared rapidly. Of 7 sera taken within 2 weeks after the lesions first appeared, 5 gave positive flocculation tests and one doubtful, and all sera from rabbits with lesions of two weeks or longer duration gave positive flocculation tests. A detectable level of immobilizing antibody appeared somewhat more slowly. During the first month, one of 10 sera was positive and three doubtful; in the second month of lesions 8 of 11 were positive and 2 doubtful; and by the third month all were positive.

Of the four animals with transient lesions during treatment, two (30–96 and 30–81) gave a positive flocculation test with a titer of one Eagle unit on a single bleeding while the transient lesions were present, but gave negative tests on later sera. A third animal (30–78) gave doubtful immobilization tests on three different sera taken after the appearance of the transient lesions but before the post-treatment lesions. With the exception of these inconclusive and transient serological tests, all the other serum specimens of these four animals gave negative tests until the post-treatment lesions appeared, and thereafter positive reactions developed in a normal sequence.

The rabbit (31–09) which developed disseminated skin lesions although the local lesions were completely suppressed, had negative flocculation and immobilizing serum reactions as late as the 73d day, or 14 days beyond the theoretical incubation period. At the time of the next bleeding on the 103d day the standard test was positive (2 Eagle units), but the immobilization reaction was still negative. Generalized skin lesions were first recognized 3 days later. They were well developed, however, and may have been present for several days.

Suppression by Penicillin—Lymph node infectivity

The popliteal lymph nodes of 8 animals in this group were removed and inoculated into the testes of normal rabbits. These transfers served as controls for the lymph nodes removed from the cured group of animals. Fifteen of the 16 passage animals developed a specific orchitis. The incubation periods in these 15 animals were 23–24 days in 3, 28–31 in 9, and 36–42 days in 3.

DISCUSSION

Theoretical results of subcurative penicillin treatment

From the evidence of prior work, cited above, it appears that the usual course of a syphilitic infection is determined by a regular logarithmic increment of treponemes which continues until sufficient are present to produce a

lesion. It also seems probable that a single dose of penicillin will destroy a percentage of the organisms present in proportion to the size of the dose. A single subcurative treatment may therefore be considered as an interruption of an orderly process which reverts to an earlier stage and subsequently resumes its usual course. If this be true, it follows that the potency of a single curative dose of penicillin given at any time during the incubation period will be measured by the prolongation of the incubation period which it induces.

Pursuing this line of reasoning, destruction of 50 per cent of the organisms at any one time would be equivalent to the deletion of one generation, and the resultant delay in incubation period would be about 33 hours which is approximately the division time for this organism (8). Similarly destruction of 90 per cent would cause a prolongation of about 4½ days; 99 per cent a delay of 9 days; 99.9 per cent a delay of 13½ days; and so forth.

The largest dose which is not curative is assumed to be one that kills all except a single organism. Maximum prolongation of the incubation period as a result of a single dose of penicillin would occur, if such a dose were given immediately before symptoms were about to appear. The theoretical limit for a single dose should therefore be equivalent to the incubation period after inoculation of a single organism. This can be calculated by extrapolation from the data of Cumberland and Turner as about 30–40 days (8).

Experimental results of subcurative penicillin treatment

In the preliminary studies presented in this paper (Chart 1) it was found that the length of the incubation period increased regularly with increasing small single doses of penicillin.

In further studies (Table 3, Chart 2) a sub-symptomatic level was maintained for periods up to 167 days by periodic intermittent treatment with penicillin. The doses used (2 and 4 mgm per kilo) were of an order which on the basis of the preliminary experiments would delay the incubation period one week, and given at weekly intervals were calculated to maintain the spirochetes at a fairly constant subclinical level. The "theoretical incubation period" as referred to frequently hereafter, has been determined by adding to the length of the mean incubation period of the untreated controls, one week for each penicillin treatment.

Since the mean incubation period in the controls was 17 days, the lesions in an animal that had received one penicillin treatment should theoretically show an incubation period of approximately 24 days; one that had received two treatments 31 days, and so on. It is interesting to note how closely the observed incubation period of most of the lesions approximated the theoretical incubation period.

The results of the experiment with repeated penicillin treatments (Tables 2 and 3; Chart 2) were altogether consistent with the theories of treponemal

growth and penicillin action outlined above. It was to be expected that the dosage would be larger than necessary for some animals, and too small for others. In order to allow for variation in individual animals two dosage levels around the calculated optimum were employed. It developed that most of the 17 animals which were cured were in the large dose group, while all 4 animals which developed progressive lesions during treatment were receiving the smaller dose. In 19 animals including 4 which showed very minimal evidence of lesions during treatment, approximately the desired level of treponemes was maintained throughout the treatment schedule.

It should be evident that it was possible to maintain this balance between spirochete and host only by a precisely calculated schedule based on the preliminary studies, as well as on the above mentioned theoretical considerations.

Evolution of disease after repression by penicillin

The subsequent course of syphilitic lesions in those animals in which the disease was successfully maintained at a subclinical level during the treatment schedule, was remarkably similar to the ordinary experimental disease. When the restraining influence of penicillin was removed, the disease process seemed to evolve very much as in untreated animals. Apparently no major change in the general immunity of the host occurred, despite the presence of treponemes for periods as much as 20 weeks longer than in parallel untreated animals. Neither Wassermann reagin nor treponemal immobilizing antibody was detected during the period of subclinical infection and these developed only after the appearance of clinically recognizable lesions.

The principal divergence from the usual course was seen in the early developmental stages of some of the initial lesions. As noted previously, in some animals multiple small papules appeared at one site and later coalesced, while in others one or more lesions failed to develop at all.

The chance localization of viable treponemes after repeated episodes of destruction may explain these bizarre lesions which were seen with increasing frequency after long periods of treatment. Likewise, in animals in which lesions developed at one site of inoculation, but failed to develop at others, all or nearly all of the treponemes may have been destroyed by the penicillin at the latter sites. On the other hand, it is possible that all of these changes may have been secondary to the development of a slight local immunity.

It is of interest to note from Table 3 that there is a progressive lengthening of the mean incubation period beyond the theoretical period in those animals which showed defective patterns. For example, of 13 animals with a complete pattern of 4 lesions, the observed mean incubation was 2.7 days longer than the theoretical; in 3 animals developing 3 lesions 5.7 days longer; while in 2 animals developing only 2 and 1 lesion respectively, the observed incubation period was 12 and 14 days longer than the theoretical period.

Generalized lesions after repression of disease by penicillin

In animals that developed generalized lesions, these lesions occurred at a remarkably uniform time interval with respect to the last penicillin treatment, which suggests that they arose as a result of the implantation of a single treponeme, or at least a very few organisms, some time after the last penicillin treatment.

Penicillin resistance

No evidence of developing penicillin resistance was observed during these studies. Even after as many as 19 different exposures to the drug at subtherapeutic levels there was no obvious change in the response to the following injections, and no increasing tendency for the treponemes to "breakthrough" and produce clinical lesions before the termination of treatment. Some animals did exhibit small lesions while still receiving treatment, but such instances tended to occur simultaneously at four sites, and early in the course of treatment, rather than late, and in most cases the lesions began to disappear during further treatment.

Implications with respect to the use of penicillin prophylactically

These studies were carried out under rigidly controlled experimental conditions—a measured inoculum of a virulent strain of treponemes, a relatively stable and favorable environment, and carefully graded and spaced doses of penicillin. Even under these circumstances entirely symptomless infections were not observed by us, and have occurred under experimental conditions only rarely if at all, in other laboratories through the use of penicillin.

The reaction of the human host to infection with *T. pallidum* under natural conditions is probably far more variable than the reaction of the rabbit. Yet these studies suggest that in man, too, entirely symptomless syphilitic infection as a result of penicillin alone is an uncommon occurrence.

It is now believed that a number of factors, prominent among which is temperature (14), influence the multiplication of treponemes and hence the clinical course of syphilis in man and animals. The action of penicillin may therefore be only one of a number of factors, which if timed in the right sequence might lead to modification of the course of the disease. It seems unlikely that a single or even several doses of penicillin, if insufficient to eliminate the infection, could in the absence of other modifying factors do anything more than cause a prolongation of the incubation period.

It is a common clinical experience to encounter patients who seem to have had a largely symptomless infection, although from a strictly biologic point of view this cannot be proved. There is no convincing evidence thus far that the wide use of penicillin in the treatment of many non-syphilitic infections

or in the prophylaxis of disease has substantially increased the proportion of persons whose syphilitic infection has been inapparent throughout.

These studies provide additional evidence that during the incubation period of syphilis the infection may be cured with minimal amounts of penicillin. The wide use of penicillin in the prophylaxis and treatment of a variety of conditions has unquestionably resulted in the cure of many syphilitic infections which were still in the incubation period. Indeed, the seeming decline in incidence of syphilis in this country since the advent of penicillin may be due in greater measure to this, rather than to its direct application in the treatment of diagnosed cases of syphilis.

Demonstration in these experiments of the uniformity of host-spirochete-penicillin interaction under carefully controlled conditions, suggests that further exploration of the spacing of penicillin dosage in relation to the multiplication rate of treponemes may provide a more rational basis for the design of maximally efficient therapeutic and prophylactic penicillin schedules.

SUMMARY

1. Single subcurative doses of penicillin prolong the incubation period of experimental rabbit syphilis in direct relationship to the size of the dose up to a limit of about 30–40 days. A single dose of 3 mgm/kilo crystalline penicillin G in aqueous solution prolonged the incubation approximately one week. Extended prolongation of the incubation period was effected by giving repeated subcurative doses (2–4 mgm/kilo) at weekly intervals.

2. In rabbits in which subclinical infection was maintained by penicillin for various periods up to 20 weeks, the maximum attempted, the observed incubation periods corresponded remarkably closely to those predicted on the basis of theoretical considerations. The evolution of the syphilitic infection after termination of treatment was in general similar to that observed in untreated animals. However, some modification was frequently noted in the appearance of the initial lesions after prolonged administration of subcurative doses of penicillin.

3. Wholly symptomless infection was not observed. Rabbits were either cured, or subsequently developed clinically recognizable lesions.

4. Serological changes were not detected during greatly prolonged incubation periods; even after 20 weeks of treatment infected animals failed to show detectable antibodies either to beef heart antigen or to *T. pallidum* as tested by the treponemal immobilization test.

5. No evidence of the development of penicillin resistance was noted in these experiments.

6. These experiments indicate that in the rabbit the prolonged presence of a relatively small number of treponemes in a syphilitic infection, which has been

maintained at a subclinical level from its inception, by repeated subcurative doses of penicillin, does not stimulate the immune mechanisms of the host sufficiently to modify in a substantial way the subsequent evolution of the disease when the administration of penicillin is discontinued.

7. The implication of these experimental results in terms of the use of penicillin in the prophylaxis and treatment of gonorrhea and other infectious diseases in man is discussed.

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PROTEIN-BINDING PROPERTIES AND CLINICAL EFFECTS OF 3-HYDROXY-2-PHENYLCINCHONINIC ACID*

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During the past 16 months, we have given approximately 18,000 tablets (3,600 grams) of 3-hydroxy-2-phenylcinchoninic acid[1] to persons with a variety of disorders. This report concerns the clinical efficacy of this drug, its toxicity and a consideration of its protein-binding properties.

Some years ago, cinchophen (2-phenylcinchoninic acid) was employed extensively in the treatment of rheumatoid arthritis and gout. It fell into disfavor when it was found that it produced severe, and frequently fatal, liver damage in an occasional individual (1). Recently, interest has turned to a derivative of cinchoninic acid, 3-hydroxy-2-phenylcinchoninic acid which henceforth will be referred to as HPC. In animals, HPC was shown to have few toxic properties when administered as a single large dose or as smaller repeated doses for long periods (2). Its pharmacologic effects include the ability to suppress renal excretion of phenol red (3), an antidiuretic action (4), an ability to stimulate the adrenal gland by way of the pituitary gland (5) and an antipyretic effect (6). A fluorometric method has been developed for the estimation of HPC in blood and urine (2). Very little unchanged drug is excreted in the urine.

Clinical studies with HPC by Blanchard and coworkers (7) suggested that HPC might be useful in the treatment of rheumatic fever. The effectiveness of HPC therapy in rheumatoid arthritis and in disseminated lupus erythematosus was not as striking but offered some promise. There was no improvement in two cases of bronchial asthma treated with HPC. Subsequently Rennie and coworkers (8) reported their results with HPC therapy in a small series of cases of rheumatic fever, periarteritis nodosa, disseminated lupus erythematosus and scleroderma. Improvement was particularly evident in the patients with rheumatic fever and in those with scleroderma. In neither the series of Blanchard and coworkers or in that of Rennie *et al.*, were any serious toxic reactions to HPC observed. Mild gastro-intestinal symptoms occurred occasionally. There was one instance of drug fever (7). With such favorable reports on HPC therapy, further studies seemed to be indicated.

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[1] The drug used in this study and a placebo resembling it were furnished through the courtesy of Dr. William P. Boger of Sharp and Dohme, Inc., Glenolden, Pa.

CLINICAL OBSERVATIONS

HPC was given to 35 patients, most of whom were considered to have one of the so-called collagen disorders. The drug was administered orally in three divided doses each following a meal. The majority of the patients were ambulatory and were seen regularly at intervals of two or three weeks.

Twenty-one patients with rheumatoid arthritis received HPC in a daily dosage ranging from 15 to 50 mg. of drug per Kg. of body weight. Eight of the 21 received more than 30 mg. per Kg. The duration of therapy was less than one month in 12 patients and, in the remaining nine, ranged from two to 16 months.

The effectiveness of HPC in the treatment of rheumatoid arthritis is difficult to assess because of its well known variation in intensity from month to month. Two patients were given alternate doses of HPC and of a placebo with the same appearance. These patients noted intensification of their discomfort while on the placebo and refused to take any more. Of the 21 arthritics studied here, ten had been observed continuously by the writer for more than three years prior to HPC therapy. All 21 patients had received sodium salicylate or acetylsalicylic acid in a dosage ranging from 1.8 to 7.2 grams per day during some part of their illness. As compared with sodium salicylate, 16 of the 21 preferred HPC. In part, this was due to the absence of tinnitus and sweating with HPC as opposed to their high incidence with salicylate administration. In part, however, the patients thought that more relief from the symptoms of arthritis occurred with HPC. There was nothing in this study to indicate that HPC appreciably alters the course of the disease. Many patients developed recurrent swelling and tenderness of additional joints while receiving HPC.

Serial determinations in these arthritics gave no indication that HPC affected the erythrocyte sedimentation rate, the volume of packed red cells or the total leucocyte count either adversely or favorably. Nine of the 21 patients at some time during the period of HPC administration were treated for several weeks or longer with ACTH or cortisone. With one exception, improvement in the disease during this period was far in excess of that noted during HPC medication.

In summary, HPC frequently relieved joint discomfort in patients with rheumatoid arthritis. It did not appear to alter the course of the disease. Its effectiveness for most patients with this disorder appeared to be greater than that of sodium salicylate administered in maximally tolerated dosage and, in addition, HPC medication was not attended by some of the unpleasant side reactions accompanying salicylate therapy. In no way did HPC approach the effectiveness of ACTH or cortisone in altering the symptoms and findings in rheumatoid arthritis.

Two patients with hypertrophic arthritis of the knee and hips were given HPC for a period of two months. Both thought that this drug was more effective than a salicylate in controlling their joint discomfort.

Three patients with acute rheumatic fever received HPC for a period of from two weeks to two months in a dosage varying between 22 and 36 mg. per Kg. of body weight. One of these patients previously had had ACTH and had relapsed when it was discontinued. Her temperature returned to normal and her joint symptoms subsided within 4 days after beginning the HPC. However, the erythrocyte sedimentation rate remained elevated for six weeks after the HPC treatment had been begun. A second patient with rheumatic fever was afebrile but had persistent electrocardiographic changes and arthralgia. Her joint symptoms subsided within four days after HPC was begun but the electrocardiographic changes remained for four weeks longer. There was no reduction in the sedimentation rate during the 60 days of HPC administration. A third patient with recurrent acute rheumatic fever and with erythema multiforme was given HPC early in the second week of her illness. During the two weeks of HPC administration, the joint symptoms subsided. However, new erythematous skin lesions continued to appear during the first week of administration of this drug and fever was present throughout the two weeks.

Four patients with disseminated lupus erythematosus took HPC tablets for periods ranging from ten to 42 days in a dosage of from 22 to 52 mg. per Kg. of body weight. In one patient there was slight subjective improvement during this period while in the other three there was no benefit.

Of the two patients with scleroderma, one improved during HPC administration and a second was unaffected. The patient who improved was a 25 year old housewife who had had scleroderma of the face, hands, forearms and upper arms for two years. During the 42 day period of HPC administration, she noticed less stiffness of the affected skin. Following discontinuation of the drug she continued to get better so that nine months later, the skin seemed normal except for patches of vitiligo which appeared in the affected skin. This favorable response during and following HPC medication was probably not related to the therapy. In the second patient there were extensive changes of long standing and in this man there was neither subjective nor objective improvement during one month of HPC treatment. Six months later he died and autopsy showed extensive visceral lesions of scleroderma.

One patient with chronic gouty arthritis was given HPC in a daily dosage of 18 mg. per Kg. of body weight for a period of six months. In the previous six months and in the four month period subsequent to HPC therapy, he has averaged one acute attack of gouty arthritis every two weeks. However, during HPC administration, no acute attack occurred.

BLOOD LEVELS OF HPC

In a few instances, blood levels of HPC were measured, using the fluorometric method described by Marshall and Dearborn (2). Ethylene dichloride ex-

traction was used to distinguish between fluorescing drug and fluorescing degradation products of the drug in the serum. The total serum levels (free plus conjugated drug) varied between 5.5 and 18 mg. of drug per 100 ml. of serum. The mean value for 15 determinations was 9.4 mg. The measurements were made several weeks to several months after initiation of medication. The highest HPC serum level (18 mg. per cent) was found in a patient taking 27 mg. of drug per Kg. of body weight. In a patient taking 50 mg. of drug per Kg. the serum level was 11.7 mg. per cent.

TOXIC REACTIONS

Skin rashes were the most common untoward effect of HPC administration. Thirteen of the 35 patients treated with HPC developed this complication. Most common was a vesicular lesion appearing on the dorsum of the hands, on the interdigital webs and on the face, particularly the nose and forehead. These lesions, at first minute vesicles, later became ulcerated and covered with heavy crusts. Usually the lesions remained inconspicuous as scattered discrete lesions. However, in three of the ten patients exhibiting this type of eruption, the lesions became confluent, forming multiple crusted areas varying from one to three cm. in greatest diameter. The time of appearance of this vesicular lesion after the beginning of drug therapy varied widely, being one week for one, two to four weeks for two, from three to six months in six and 12 months in one. Drug administration was stopped in all patients within a week after the onset of the rash, which then disappeared within one to two weeks. Five patients with rheumatoid arthritis in whom HPC had afforded good symptomatic relief resumed HPC at a subsequent period after having had a previous vesicular rash. In each of these the rash returned within three to five days after resumption of HPC. Nonetheless these patients have continued to take this drug for many months after the recurrence of the rash and it has not increased in extent. Certainly this type of rash would appear to be an insignificant complication in the majority of cases.

The relation of this vesicular eruption to exposure to sunlight is unclear. Many patients receiving HPC have observed itching and burning of the skin after exposure to sunlight. In six of the ten patients with vesicular lesions, the onset of the eruption was associated with exposure to sun. However, it is difficult to understand how this rash can be confined to a few fingers or confined to the bridge of the nose when an area so much greater is exposed to sunlight. Moreover, the prompt recurrence of the rash after resumption of the drug was not associated with exposure to sunlight. Three of the ten patients with this rash noted its onset in midwinter, at a time when there was relatively less sunlight.

One patient developed a diffuse papular erythematous rash on the thighs and upper parts of the lower legs. This appeared one week after HPC administration and vanished within ten days after the drug was discontinued.

Another patient, after three months of HPC medication, developed a scaly erythematous rash on both upper arms. The eruption was confluent and was accompanied by intense itching. Later this spread to involve the forearms but not the hands. Five weeks after HPC medication was discontinued, new lesions still were appearing on the forearms. Most of this rash had disappeared, however, within nine weeks after the drug was stopped.

Five weeks after HPC medication had been stopped a sample of the patient's serum was transferred to the skin of two control subjects. Twenty-four hours later, HPC was injected into the control subjects at the site of serum administration as well as in a previously untouched area. In both control subjects, marked erythema and wheal formation appeared at the site of the passive serum transfer but not in the skin areas where only HPC was injected.

Finally, on one patient intense diffuse erythema of the face and ankles appeared ten days after HPC therapy had been begun. The patient ascribed the facial erythema to exposure to sun and the ankle erythema to application of a heat lamp. In spite of continued drug administration, the erythema of both the face and ankles disappeared over a period of one week.

Eight of the 35 patients developed diarrhea while on HPC medication. In only three was this symptom severe enough to cause discontinuation of the drug. Two patients, after many months of HPC medication, developed epigastric burning which persisted for several weeks and then disappeared with continued drug administration.

One patient with disseminated lupus erythematosus had chills, fever and malaise which began 48 hours after taking HPC. She stopped this drug promptly and her symptoms subsided within 24 hours. Three days later she resumed medication with HPC and 12 hours later she again had chills and fever which lasted only a day. Each time herpes febrilis lesions appeared on the lips. It seems reasonable to assume this to be a drug fever. A second patient complained of intense muscular stiffness after taking HPC for 48 hours. This continued for three days after the drug was stopped.

Only one patient developed tinnitus while taking HPC. No patient developed edema. In one patient with rheumatoid arthritis and mild congestive heart failure, administration of HPC for several weeks did not aggravate his edema even though digitalis, mercurial diuretics and salt restriction were purposely withheld. In this patient plasma volume estimation with T 1824 before beginning HPC was not significantly different from the results of a plasma volume estimation on the 14th day of HPC medication.

In all patients serial studies were made of the erythrocyte sedimentation rate, the packed red cell mass, the total leucocyte count and the differential count. There was no observed change which could be ascribed to HPC. Repeated direct eosinophil counts on four patients during many months of HPC administration showed no decrease as compared to eosinophil levels prior to therapy. Serial estimations by chemical procedures of fibrinogen, serum gamma

globulin, albumin and serum mucoprotein were carried out on six patients. While fluctuations in values were observed, the decrease in gamma globulin and in plasma fibrinogen and the rise in serum albumin noted so frequently in patients receiving ACTH could not be demonstrated in the patients receiving HPC (9).

PROTEIN-BINDING PROPERTIES OF HPC

Marshall and Dearborn (2) indicated that more than 95 per cent of the HPC in serum is bound to the serum proteins. It seemed of interest to determine which of the serum proteins might be responsible for this protein-binding. It was thought that some information might be obtained from *in vitro* studies. Accordingly, the following experiment was performed. A five ml. aliquot of fresh serum from a normal subject and similar amounts of serum albumin, serum gamma globulin (fraction $II_{1,2}$) and serum mucoprotein[2] (10) were dialyzed for 24 hours against three changes of 0.02 M. phosphate buffer at pH 7.3. Sufficient sodium chloride was added to the buffer to make it isotonic with 0.90 grams per cent sodium chloride (W/V). The protein solutions in viscose sausage casings then were suspended in a beaker containing 700 ml. of the same buffer to which had been added HPC to a concentration of approximately 10 mg. per cent. The beaker in which the protein bags were suspended was allowed to rotate at 100 r.p.m. for three hours at a temperature of 31° C. The protein solutions in the bags were then examined for HPC content, pH and for protein concentration. The HPC level of the non-protein containing fluid in the beaker was found to be 9.3 mg. per cent at the end of the dialysis experiment. The pH of the protein in the bags was 7.2 $\pm$ 0.1. The other findings are given in Table I. It will be observed that both human serum and serum albumin were quite effective as binding agents for HPC. The mucoprotein was less effective while the gamma globulin was extremely poor in its binding capacity. For comparative purposes, the moles of HPC bound per mole of protein have been calculated. The fact that the binding effect of mucoprotein with HPC was more evident after the supernatant is treated with heat and HCl is of considerable interest. This vigorous treatment did not lead to precipitation of the mucoprotein from solution. The possible significance of this observation will be discussed subsequently. It should be pointed out that the protein concentrations used in this *in vitro* study are not comparable to the concentrations of these proteins as they usually exist in human serum. It seemed desirable to use equal concentrations of each protein component for the comparison. Unfortunately, there was an insufficient supply of mucoprotein to obtain a concentration as great as that employed for

[2] The serum mucoprotein was supplied through the courtesy of R. J. Winzler. It is a part of the sample used in a study of certain physical characteristics of this protein, as described in reference 10.

the other proteins. In order to apply these protein-binding values to the conditions present in human serum, it would be necessary to carry out additional experiments, using varying drug concentrations and varying protein concentrations (11).

In order to gain some approximation of the tightness of the bond for HPC with serum proteins, two ml. of fresh human serum to which HPC had been added, were dialyzed with a rotating membrane for 3 hours at pH 7.3 against 700 ml. of the 0.02 M. phosphate buffer containing sodium chloride. The HPC level of the serum prior to dialysis was 9.6 mg. per cent and after dialysis, was 9.2 mg. per cent. This suggests that HPC was bound firmly to the serum proteins under the conditions employed. In this respect, the situation differs significantly from salicylate which also binds with serum proteins but, under the same circumstances, can be removed by dialysis.

TABLE I

Protein-binding Capacity of HPC for Proteins in Vitro

SUBSTANCE IN DIALYSIS BAG	PROTEIN CONTENT GM. PER CENT	HPC LEVEL (FREE) MINUS HPC LEVEL OF DIALYSATE	HPC LEVEL (TOTAL)* MINUS HPC LEVEL OF DIALYSATE	MOLES HPC BOUND PER MOLE OF PROTEIN
		mg. per cent	*mg. per cent*	
Human Albumin	2.85	39.0	39.3	3.6
Gamma Globulin	3.00	2.4	2.4	0.5
Mucoprotein	0.75	2.6	6.7	1.5
Human Serum	3.00	31.5	31.8	

* Total HPC level determined by treatment of supernatant after metaphosphoric acid precipitation with HCl and heating to 100° C. for 30 minutes.

Some approximation of the protein-binding capacity of HPC *in vivo* might be obtained from comparative levels of the HPC content of the serum and spinal fluid. Spinal fluid was obtained from a patient who had been maintained for two weeks on HPC in a daily dosage of 32 mg. per Kg. of body weight. At the time of withdrawal of the spinal fluid, the serum HPC level was 14.2 mg. per cent for free drug and 15.0 mg. for total drug. The HPC level on the spinal fluid of this patient was 0.7 mg. per 100 ml., there being no conjugated drug. These values are in keeping with the concept that nearly all of the HPC in the blood is bound to serum proteins.

Marshall and Dearborn (2) found that a part of the HPC in the serum was present in a conjugated form. This could be detected by treating the supernatant of serum to which metaphosphoric acid (HPO_3) had been added to a final concentration of 4 per cent, with a small quantity of concentrated HCl and then heating the solution for 30 minutes in boiling water. The nature of this conjugated drug is intriguing. At present the best hypothesis would appear to be that the conjugated drug in the metaphosphoric acid supernatant actually

represents drug bound to serum mucoprotein. Evidence to support this hypothesis is derived from the experiments considered below.

When serum mucoprotein determinations were performed on the sera of patients receiving HPC, it was observed that the precipitated mucoprotein had an intense yellow color instead of its usual white color. This might mean that HPC was bound to mucoprotein. In carrying out the mucoprotein estimation by the method of Winzler and coworkers (12), 2.0 ml. of undiluted serum are treated with 8.0 ml. of 0.75 M. perchloric acid. This precipitates most of the serum protein. The supernatant is treated with 5 per cent phosphotungstic acid in 2 N. HCl. The protein precipitated by this reagent is regarded as mucoprotein.

The serum mucoprotein on a sample of serum from a patient with rheumatoid arthritis was found by this method to be 179 mg. per 100 ml. To a similar sample of 2.0 ml. of undiluted serum were added 8.0 ml. of 5 per cent metaphosphoric acid. The supernatant was treated with the phosphotungstic acid and was found to have a protein content of 652 mg. per 100 ml. It would seem, therefore, that this concentration of metaphosphoric acid does not precipitate all the protein from serum. The characteristics of mucoprotein are such that it would not be expected to be in the precipitate; the resistance of serum mucoproteins to precipitation with such agents as sulfosalicylic and trichloracetic acids is well known (13). Certain serum mucoproteins may be obtained in the supernatant after heat coagulation of other proteins (13). Similarly, as noted earlier, metaphosphoric acid in a final concentration of 4 per cent did not precipitate a 0.75 Gm. per cent solution of pure mucoprotein dissolved in 0.85 Gm. per cent sodium chloride. Moreover, addition of a small quantity of HCl to the solution of mucoprotein in HPO_3 and heating to 100° C. for 30 minutes did not precipitate this protein. As is indicated in Table I, a solution of mucoprotein to which HPC has been added gives greater fluorescence and a higher HPC content after boiling with addition of a small quantity of HCl. This is comparable to the increase in HPC (conjugated drug) found after heating the serum of human subjects who have been receiving HPC.

The immediate objection that may be raised to the claim that conjugated drug represents drug bound to mucoprotein is the observation of Marshall and Dearborn (2) that when a known amount of HPC is added to a known volume of serum, the total drug concentration may be calculated accurately by estimating the HPC level of this serum treated only with HPO_3 and not subjected to the addition of HCl and heating. We have confirmed this finding on three occasions. In explanation of this seeming contradiction, it might be suggested that some time must elapse before a firm bond occurs between mucoprotein and HPC. Serum from a patient with Hodgkin's disease in which the serum mucoprotein was 530 mg. per 100 ml. (normal mean value is 87 mg.) was incubated for three hours at 37° C. with HPC. At the end of this period,

the free HPC level of this serum was 9.6 mg. per cent while the total HPC level was 13.1 mg. per cent. The determinations were done in quadruplicate with good agreement between individual values.

DISCUSSION

The clinical studies presented here serve to amplify the observations previously reported by Blanchard and coworkers (7) and Rennie *et al.* (8). In agreement with these reports we found HPC to be a useful agent in the treatment of many cases of rheumatoid arthritis. It is more difficult to draw conclusions concerning its place in the treatment of rheumatic fever. One of the three patients with acute rheumatic fever presented in this study failed to show a response to HPC. Even in the light of the favorable results in the previous two reports, the total number of cases treated is too meager to permit any conclusion. The disease, rheumatic fever, varies greatly in duration. It may well be found after adequate trial that HPC is not superior to sodium salicylate in this disorder.

The treatment of disseminated lupus erythematosus with HPC did not appear to be of striking benefit in the four cases treated here. Similarly in the two reports already cited, the favorable effects in this disorder were not impressive. The marked improvement noted in some of the cases of scleroderma treated by Rennie and coworkers must be considered with reservation until additional observations are made. Like rheumatoid arthritis, this disease fluctuates in intensity and spontaneous remissions are not infrequent.

Thus, it is difficult to evaluate at this time the effectiveness of HPC in the collagen disorders as a whole. It would appear that the primary effect is symptomatic improvement with relief of discomfort but without any real alteration in the disease process. This is supported by the fact that laboratory evidences of improvement during HPC therapy have not been impressive.

The high incidence of toxic skin reactions observed by us is at variance with the two earlier reports. It must be pointed out that many of our patients were ambulatory. This emphasizes the possible role of exposure to sunlight in the development of skin rashes. Moreover, the duration of therapy in nearly half of our patients extended into many months whereas, in the previous studies, therapy usually was of shorter duration. In agreement with previous accounts, no serious toxic reactions occurred. Diarrhea was the most common complaint apart from skin lesions.

Only a few of the protein-binding properties of HPC have been studied. As in the case of certain other drugs, albumin seemed to have the greatest binding capacity for HPC (11). The ability of HPC to bind serum mucoprotein is of considerable interest since this may account partially or wholly for the conjugated drug present in human serum.

SUMMARY

HPC (3-hydroxy-2-phenylcinchoninic acid) was given to patients with rheumatoid arthritis, rheumatic fever, scleroderma and disseminated lupus erythematosus. In arithritis, it frequently gave more symptomatic relief than could be achieved by salicylates. In the other disorders, it was not impressively effective in the cases studied.

Diarrhea and skin eruptions were the most common toxic manifestations. No serious reactions were observed.

Studies on the protein-binding properties of HPC are presented. *In vitro*, serum albumin possessed greater protein-binding ability for HPC than did serum gamma globulin or serum mucoprotein.

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CLINICAL USE OF THE ARTIFICIAL CIRCULATION WITH A NOTE ON INTRA-ARTERIAL TRANSFUSION*

A. MARIO DOGLIOTTI†

Received for publication November 14, 1951

I feel greatly honored in having extended to me the opportunity of addressing the group of outstanding surgeons gathered here this evening. I am especially glad to be here with Dr. Blalock who was so kind as to come to Turin recently to inaugurate our center for cardiac surgery which has been named in his honor. I would like to take this occasion to say again how much we appreciated his visit, and I would like to present to Dr. Blalock an album of photographs as a souvenior of his first trip to Italy.

This evening I would like to present to you a description of two procedures that have been devised in my clinic by myself with the valuable collaboration of Dr. Costantini and others among my associates. These procedures are designed for the emergency treatment of some of the more serious cardiovascular accidents. The first, on motion picture film, illustrates a technique of intra-arterial blood transfusion utilizing the common carotid artery. The fundamental principle of this technique is that the injection of blood is directed in the artery towards the brain and not towards the heart as is commonly practiced in the usual methods of intra-arterial transfusion. Our contention is that it is more urgent and of more vital importance to maintain or to restore an adequate blood flow to the cerebral centers than to the heart itself. There is a considerable body of clinical evidence to support the view that after three to five minutes of cardiac arrest irreversible changes in the cerebral centers take place while the heart may regain its function after considerably longer periods of arrest. The surgical literature abounds with cases of successful cardiac resuscitation but all too many of these patients have demonstrated signs of permanent cerebral damage.

(MOVIE: technique of intra-arterial blood transfusion using the common carotid artery with the injection directed toward the brain.)

The second procedure about which I would like to say a few words concerns a mechanical method for sustaining circulatory and respiratory functions. Many investigators, including Gibbon, Crafoord, Dennis, Jongblood, Thomas Tosatti, and others, have recently carried out extensive experimental work with special apparatus which is designed to substitute for all cardiac and re-

* Presented at the Surgical Staff Conference, Johns Hopkins Hospital, October 30, 1951.

† Professor of Surgery, University of Torino, Italy.

spiratory functions. To my knowledge none of these investigators has yet used his apparatus successfully in the human being.

The apparatus that Costantini and I have constructed in the Surgical Clinic of Torino has a more limited scope. That is to say, its primary function is not that of complete substitution for the heart and lungs but rather to serve to complement activity of the heart and lungs during periods of failure of cardio-respiratory function. As may be seen in the accompanying lantern slides, our apparatus has two independent pumps. One of these pumps sucks the blood from a vein while the other injects it into an artery. The blood which is removed from the patient is heparinized and is passed through a special oxygenator which consists principally of a large porcelain filter over which a thin film of blood is passed and brought in contact with finely dispersed gaseous oxygen. The excess carbon dioxide in the blood is adequately exhausted by vacuum extraction. The newly oxygenated blood is then pumped back into one of the patient's systemic arteries.

The excessive heparin may be neutralized at any time by the injection of an adequate dosage of protamine sulfate. In order to reduce the amount of heparin required every part of our apparatus (rubber tubes, porcelain filter, glass container, plastic cannulae, etc.) is treated with silicone which, as is well known, delays the coagulation of blood in a remarkable manner.

After long experimental preparation with laboratory animals, principally dogs, we have recently used this apparatus successfully in a patient who was undergoing an intrathoracic procedure in Torino. To my knowledge this is the first reported case in which the artificial extracardiac circulation has been successfully used in man. The details of this procedure were recently reported by myself to the Paris Congress de Societé Internationale de Chirurgie (September, 1951). A short resume of this case should be of interest to you.

A. M., a 49 year old white male, was admitted to the Surgical Clinic of Torino with a diagnosis of mediastinal tumor. X-ray examination showed that the tumor occupied over half of the upper right thorax and mediastinum. On August 9, 1951 operation was carried out by myself. Under endotracheal cyclopropane-ether-oxygen anesthesia the right chest was entered. As soon as the pleura was opened the patient's condition deteriorated. Signs of marked venous engorgement of the cervical veins were evident and the patient became cyanotic. Peripheral blood pressure was unobtainable. It was felt that the failing cardiac function was probably due to a sudden increase in the degree of compression of the superior vena cava and right auricle by the huge mediastinal tumor. At this point we decided to utilize our apparatus which had been previously prepared and was available in the operating room. A long special plastic cannula was inserted in the superior vena cava by way of the right axillary vein. Another

cannula was passed in towards the aorta through the right axillary artery. These cannulae were attached by tubing to our pump oxygenator and after a few minutes of artificial circulation at the rate of about one liter per minute the patient's condition improved considerably. It was possible then to continue with the surgical procedure; the tumor was completely removed and the patient made an uneventful convalescence. At the present time he is still alive and well. The artificial circulation in this first case was maintained for 20 minutes.

THE EFFECT OF CORTISONE AND ACTH ON OCULAR INFLAMMATION SECONDARY TO THE INJECTION OF IRRITANT SUBSTANCES*

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The Wilmer Institute of the Johns Hopkins University and Hospital

Received for publication November 19, 1951

In a previous communication it was reported that the parenteral injection of cortisone in a rabbit for a period of three days before the anterior chamber injection of such irritants as jequirity and glycerin completely or almost completely blocked the ocular inflammation resulting from such injection. It was also reported that if cortisone was injected in the anterior chamber at the same time the jequirity was administered, that it had a similar blocking effect. The purpose of this paper is to report further observations on the blocking effect of both cortisone and ACTH on the inflammation produced by irritants. The irritants used in these experiments were jequirity infusion and staphylococcus toxin.

THE BLOCKING EFFECT OF PARENTERALLY ADMINISTERED CORTISONE AND ACTH

Jequirity. When infusions of jequirity are instilled in the conjunctival sac they produce an intense sterile conjunctivitis, characterized by chemosis, inflammation and a muco-purulent discharge. When injected in the anterior chamber, strong jequirity infusions produce a violent uveal and corneal inflammation, with actual tissue necrosis and early rupture of the globe. Weaker infusions produce pericorneal congestion, marked and continued iris inflammation, and an opacification of the cornea, affecting first the endothelium and later the deep stroma.

The only preparation of jequirity available is a somewhat coarse powder, the crude, ground-up, whole bean. Peculiarly enough, different infusions of this powder although prepared under identical circumstances, have a widely different effect. It is necessary, therefore, in any experiment to standardize the individual infusion used for its irritant action. The infusion used in these experiments was prepared by grinding up 100 mgms. of the crude powder, taking it up in 100 cc. of normal salt solution and centrifuging to remove the larger particles. Various dilutions of this primary infusion were then prepared and 0.1 cc. injected in the anterior chamber of the eyes of a series of rabbits and the eye observed for the inflammatory reaction resulting. The results of

* This work was supported by a grant from the National Institute of Health, Federal Security Agency, Public Health Service.

this tabulation are shown in Figure 1. Infusions of 1:2000, the strength used in the previous experiments caused rapid necrosis and early rupture of the globe. Dilution of 1:4000 caused a slightly less severe reaction. Dilutions of 1:8000,

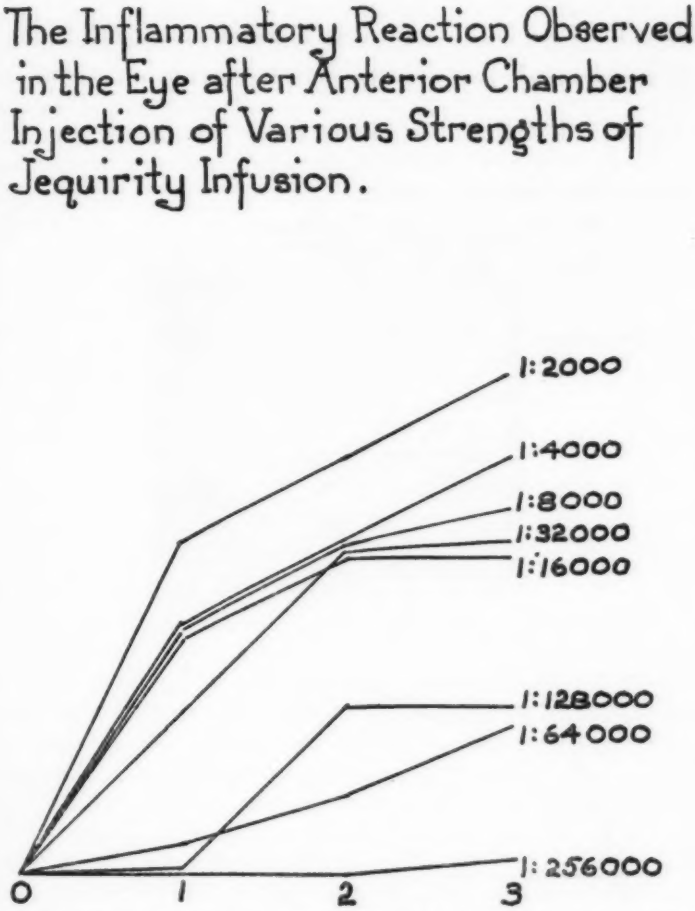

FIG. 1. The ocular inflammatory reaction produced by various strengths of jequirity infusion.

1:16,000 and 1:32,000 still caused intense reactions with occasional rupture of the globe. Dilutions of 1:64,000 and 1:128,000 caused a satisfactory inflammatory response with moderate corneal clouding. Dilutions of 1:256,000 caused only a minimal reaction.

PLAN OF EXPERIMENTS

All animals were pre-treated by the parenteral injection of 10 mgms. of either cortisone, beef ACTH, or pork ACTH for three days prior to the anterior

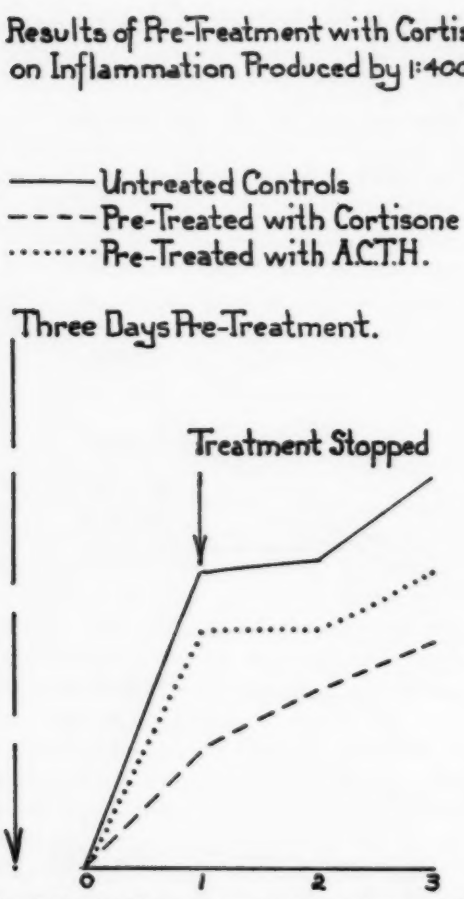

FIG. 2. Results of pre-treatment with cortisone and ACTH on ocular inflammation produced by 1:4000 jequirity infusion.

chamber injections of the jequirity. In the first two experiments, to provide a severe test for the efficacy of these agents in blocking inflammation, strong infusions of jequirity were used. In Experiment III in order to provide a less severe test, weaker dilutions of jequirity were used.

Experiment I

Twelve normal rabbits, of either sex and the same breed used in former experiments were divided into three groups of four each. Group I was left untreated as controls. Group II received cortisone, 10 mgms. per diem for 3 days prior to the anterior chamber injection and for 1 day thereafter. Two animals of Group III received beef ACTH and the other two pork ACTH, in ACHTAR vehicle, 10 mgms. per diem in divided doses for a similar period. After the pre-treatment period of Groups II and III, all twelve animals received 0.1 cc. of a 1:4000 jequirity infusion in the anterior chamber. The animals were kept under close observation for a post-injection period of 3 days. Figure 2 shows the result of this experiment. It is quite evident that these agents had

FIG. 3. Untreated control eye injected with 1:4000 jequirity infusion

only a very slight effect in controlling the severe inflammatory reaction produced by the 1:4000 strength infusion used in this experiment. The reaction in the control eyes was, however, most intense, one of the eyes rupturing on the third day and two other control eyes being on the point of rupture. Figure 3 shows a typical control eye, and Figure 4 an eye in which the reaction was slightly blocked by pretreatment with cortisone, and Figure 5 a similar eye in an animal pre-treated with ACTH.

It was quite evident that the irritant used in this experiment was too severe a test for any inhibitory action cortisone and ACTH might exert on the ocular inflammation, and accordingly the experiment was repeated with a weaker infusion.

Experiment II

This was an exact repetition of Experiment I with the single exception that the irritant used was a 1:8000 dilution of the same infusion.

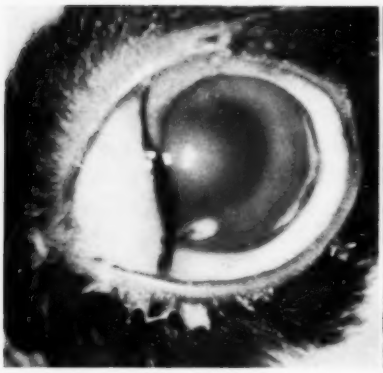

FIG. 4

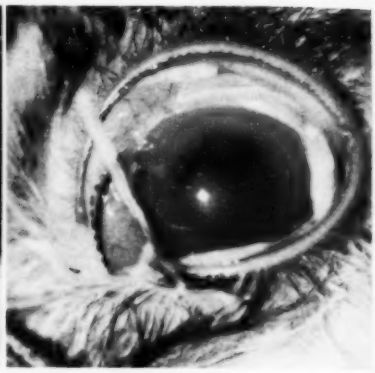

FIG. 5

FIG. 4. Cortisone treated, eye injected with 1:4000 jequirity infusion. Partial block.
FIG. 5. ACTH treated, eye injected with 1:4000 jequirity infusion. Partial block.

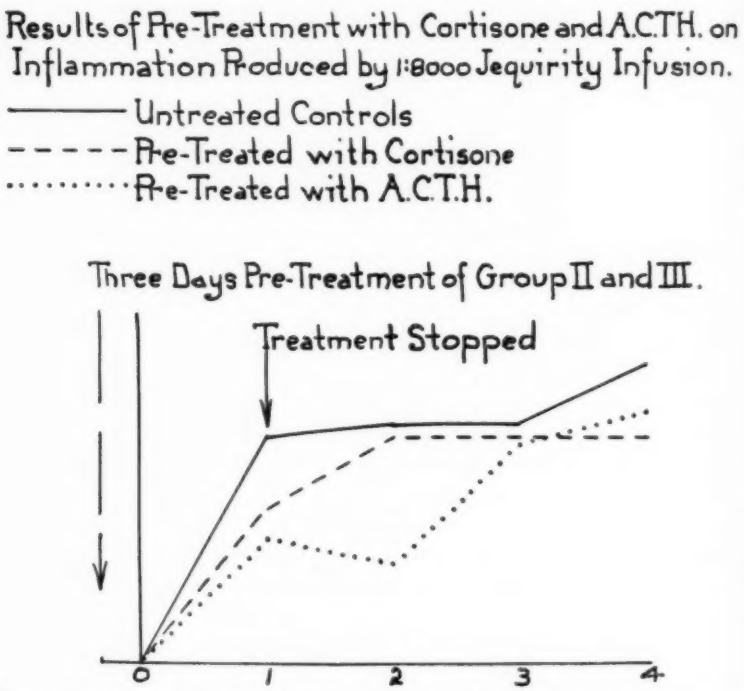

FIG. 6. Results of pre-treatment with cortisone and ACTH on ocular inflammation produced by 1:8000 jequirity infusion.

Results

The results are shown in Figure 6. Again, both cortisone and ACTH had only a slight inhibitory effect on the inflammatory response to the irritant. Such an inhibition as was noticed was exerted chiefly against the vascular reaction, peri-corneal and conjunctival injection and chemosis. The clouding of the corneal endothelium was not influenced. The reaction in the eyes of the untreated controls was only slightly less than that observed with the 1:4000 strength infusion.

This experiment clearly indicated that even 1:8000 jequirity infusion produced an ocular reaction so violent that it was beyond control through pre-

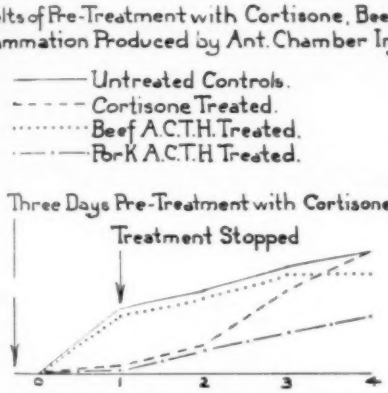

FIG. 7. Results of pre-treatment with cortisone and ACTH on ocular inflammation produced by 1:128,000 jequirity infusion.

treatment with either cortisone or ACTH. In order to provide a more fair test for the efficacy of these agents in blocking inflammation due to irritants, the above experiments were repeated using 0.1 cc. of a 1:128,000 jequirity infusion as the irritant. This strength infusion produced an intense pericorneal congestion, signs of iris inflammation, and slight clouding of the cornea, but without any evidence of necrosis or tissue destruction.

Experiment III

A group of 16 rabbits of either sex and the same race as in the previous experiments were divided into four groups of four rabbits each. Group I was left untreated as controls. Group II was pre-treated for three days with cortisone 10 mgms. per diem in divided doses, and treatment continued for one day after the anterior chamber injection. Group III was similarly treated with beef ACTH, in ACHTAR vehicle, 10 mgms. per diem in divided doses, and Group IV similarly treated with pork ACTH.

Results

The results are shown in Figure 7. All controls developed an acute ocular inflammation characterized by marked ciliary congestion, dilatation of vessels of the iris and occasionally slight fibrin in the anterior chamber (Fig. 8).

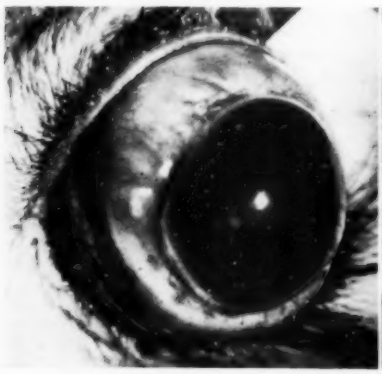

FIG. 8

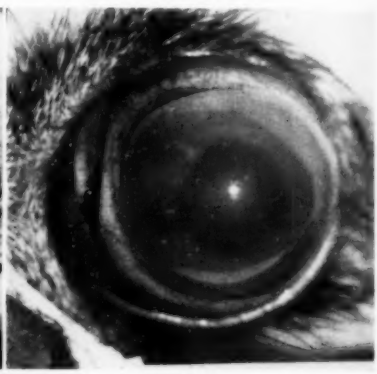

FIG. 9

FIG. 8. Untreated control eye injected with 1:128,000 jequirity infusion
FIG. 9. Beef ACTH treated, eye injected with 1:128,000 jequirity infusion. No block.

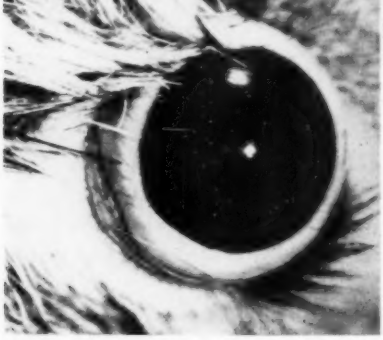

FIG. 10

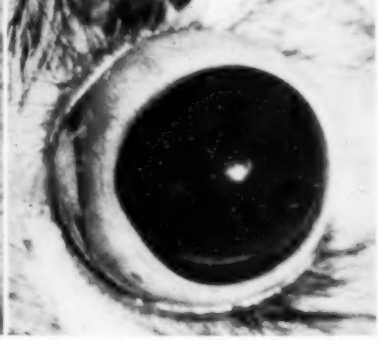

FIG. 11

FIG. 10. Pork ACTH treated, eye injected with 1:128,000 jequirity infusion. Complete block.
FIG. 11. Cortisone treated, eye injected with 1:128,000 jequirity infusion. Complete block.

The rabbits pre-treated with beef ACTH, showed an exactly similar picture (Fig. 9), the beef ACTH having no apparent blocking in the rabbits' eye. On the other hand, the rabbits pre-treated with pork ACTH (Fig. 10) or cortisone (Fig. 11) showed a complete temporary blocking of the inflammatory reaction

during the one day post-injection period during which ACTH and cortisone were continued. During this twenty-four hour period when the eyes of all the controls showed acute inflammation, the eyes of the pork ACTH or cortisone treated rabbits remained white and entirely free of inflammation. The cornea of one rabbit, (#5) pretreated with cortisone, showed a haze in the endothelial surface of the cornea, the corneas of the other seven pre-treated rabbits remained entirely clear.

When treatment with ACTH and cortisone was stopped twenty-four hours after the injection, the eyes of the cortisone and pork ACTH rabbits began to show a low-grade ciliary congestion and haziness of the cornea. This gradually increased, and at the end of four days, three days after all treatment was terminated, there was little difference between the eyes of the control and the treated rabbits. The blocking effect of the ACTH and cortisone ceased shortly after termination of treatment and since the irritant was still present in the anterior chamber, the eyes now reacted.

Staphlococcus Toxin. Staphylococcus toxin, prepared by the method described by Burky* has a definitely irritant action when injected in the anterior chamber of adult rabbits. To bring additional proof of the blocking action of cortisone and pork ACTH on inflammation due to irritants, the same experiment above outlined was repeated, this time using an anterior chamber injection of 1:50 staphylococcus toxin as the irritant.

Experiment IV

Sixteen rabbits were used in this experiment, divided again into four groups, of four each. Group I was left untreated as controls, while Groups II, III and IV were pre-treated for three days with cortisone, beef ACTH, and pork ACTH respectively in the doses described above. Treatment was continued for 24 hours after the anterior chamber injection.

Results

The results are shown in Figure 12. The anterior chamber injection of 0.1 cc. of 1:50 dilution of staphylococcus toxin produced a mild iritis of two days duration in the eyes of untreated controls (Fig. 13). The reaction was distinctly less and of much shorter duration than the reaction produced by the weakest jequirity infusions. Pre-treatment with cortisone completely blocked this reaction in the eyes of the treated animals. Not only was there no initial reaction, but there was no delayed reaction (Fig. 14).

Pre-treatment with beef ACTH had a slight blocking effect in two rabbits and no effect in two. In the eyes where no blocking effect was noted the iritis resulting from the anterior chamber injection of staphylococcus toxin was of

*Burky, Earl L.: Production of Lens Sensitivity in Rabbits by the Action of Staphylococcus Toxin. Pro. Soc. Ex. Biol. and Med. **31,** 445–447, 1933–34.

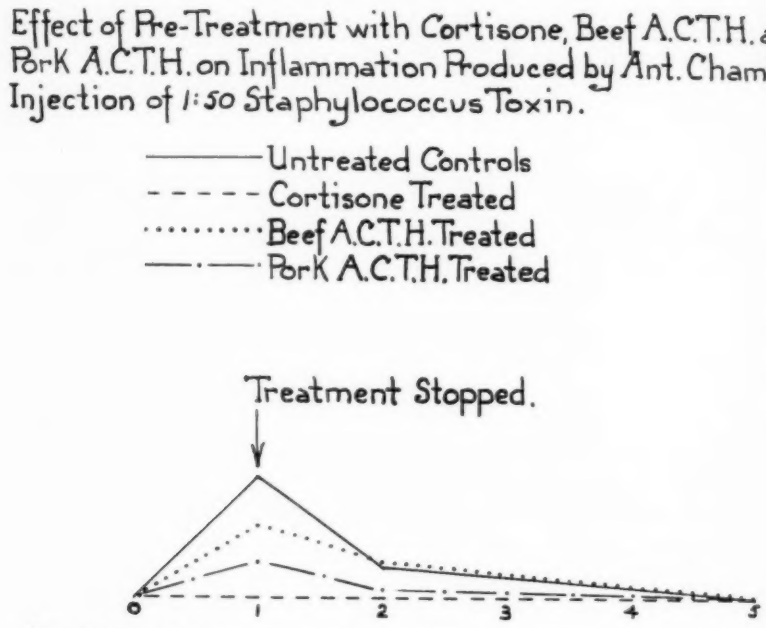

FIG. 12. Results of pre-treatment with cortisone and ACTH on ocular inflammation produced by staphylococcus toxin (1:50).

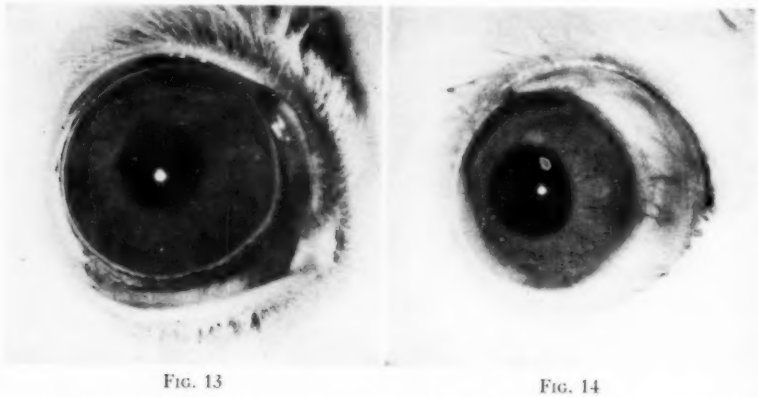

FIG. 13 FIG. 14

FIG. 13. Untreated control, eye injected with staphylococcus toxin
FIG. 14. Cortisone treated, eye injected with staphylococcus toxin. Complete block.

equal intensity to that shown by the untreated controls. In the two rabbits in which there was a partial block of the inflammation, one rabbit showing a slight reaction of one days duration while the other showed a similar reaction of ten days duration. Figure 15 shows the eye of one of these rabbits.

Pre-treatment with pork ACTH gave a much stronger blocking effect than did similar pre-treatment with beef ACTH. On the first day all four of the rabbits receiving pork ACTH showed minimal reactions. On the second day, this minimal reaction had faded in three of the four rabbits, while the last showed only a trace of inflammation. The following day all eyes were quiet and free of inflammation. Figure 16 shows the eyes of one of these rabbits 24 hours after the anterior chamber injection.

THE LOCAL ACTION OF ACTH AND CORTISONE

These experiments were undertaken (a) to confirm our previous observation on the ability of cortisone applied locally to block the inflammation produced by the anterior chamber injection of jequirity, (b) to determine if it exerted the

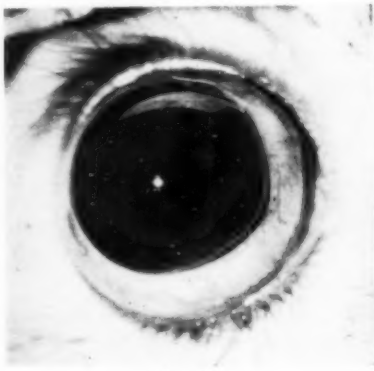

FIG. 15

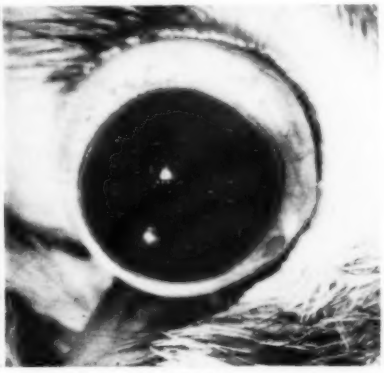

FIG. 16

FIG. 15. Beef ACTH treated, eye injected with staphylococcus toxin. Partial block.
FIG. 16. Pork ACTH treated, eye injected with staphylococcus toxin. Complete block.

same effect on inflammation produced by staphylococcus toxin, and (c) to investigate the possibility of any local action of ACTH.

As a preliminary to the first experiment, 2.5 mgms. of beef and pork ACTH in 0.1 cc. saline suspension were injected in the anterior chamber of the eyes of normal rabbits. The ACTH dropped to the bottom of the anterior chamber where it was very slowly absorbed over a 48–72 hour period. There was no inflammatory reaction in these eyes. When a similar amount of cortisone was likewise injected in normal animals, the cortisone was quickly absorbed, not a trace being seen after 24 hours. Cortisone likewise produced no inflammatory reaction.

Experiment V

Fourteen normal rabbits (of mixed race and the same breed used in the previous experiments) were used. Four rabbits (Group I) received 0.1 cc. of a

1: 128,000 infusion of jequirity in the anterior chamber. Four rabbits (Group II) received 0.1 cc. of a 1:64,000 infusion of jequirity together with 2.5 mgms. of cortisone in the anterior chamber. Two rabbits (Group III) received 0.1 cc. of a 1:128,000 jequirity infusion together with 2.5 mgms. of beef ACTH in the anterior chamber, while the remaining two rabbits received 0.1 cc. of a 1:128,000 jequirity infusion together with 2.5 mgms. of pork ACTH. Cortisone was therefore subjected to a more severe test than ACTH.

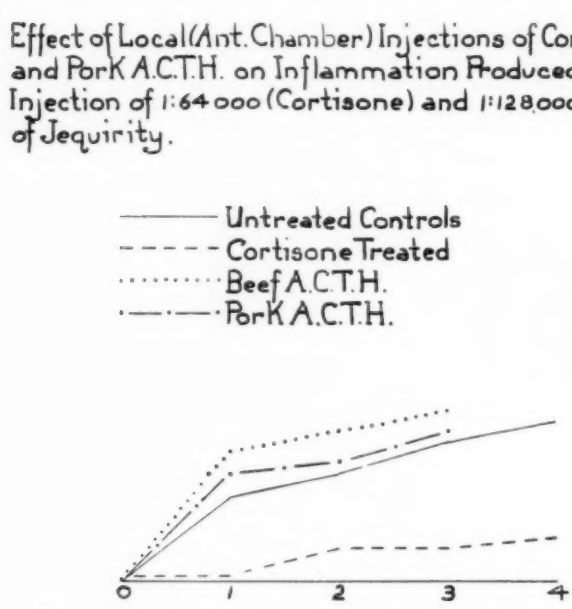
Effect of Local (Ant. Chamber) Injections of Cortisone, Beef A.C.T.H., and Pork A.C.T.H. on Inflammation Produced by Ant. Chamber Injection of 1:64 000 (Cortisone) and 1:128,000 (A.C.T.H.) Infusions of Jequirity.
Untreated Controls
Cortisone Treated
Beef A.C.T.H.
Pork A.C.T.H.
0
1
2
3
4

FIG. 17. Results of anterior chamber injection of cortisone and beef and pork ACTH combined with jequirity infusion compared to similar injection of jequirity alone.

Results

The results are shown in Figure 17. The controls developed an acute iritis, the inflammation remaining unchanged over a four day period of observation. The rabbits treated with both beef and pork ACTH developed an exactly similar iritis, of equal intensity and duration, neither the beef or pork ACTH having any blocking effect when administered topically. The rabbits injected with jequirity plus cortisone, however, behaved quite differently. After twenty-four hours, when the other rabbits all showed an acute iritis, the eyes of the animals receiving cortisone were white and free of inflammation. At the end of the second day, one of the cortisone treated rabbits showed an iritis of "1" intensity, while the other three rabbits were still entirely inactive. At the end

of the third day, a second cortisone treated rabbit showed a slight iritis. The average of the entire group was "0.3" against "1.3" for the controls and 1.4 and 1.5 for the rabbits receiving ACTH plus jequirity.

Experiment VI

This experiment was an exact repetition of Experiment V with the exception that 0.1 cc. of 1:100 staphlococcus toxin was used as the irritant instead of jequirity infusion. This was undertaken to determine if ACTH applied topically would have any effect in a less severe test. The rabbits used in this experiment

Effect of Local (Ant. Chamber) Injection of Cortisone, Beef A.C.T.H. and Pork A.C.T.H. on Inflammation Produced by Ant. Chamber Injection of 0·1 cc 1:100 Staphylococcus Toxin.
Untreated Controls
Cortisone Treated
Beef A.C.T.H. Treated
Pork A.C.T.H. Treated
0
1
2
3

FIG. 18. Results of anterior chamber injection of cortisone and beef and pork ACTH combined with staphylococcus toxin, compared with similar injection of staphylococcus toxin alone.

were smaller and younger than in the previous experiments and developed a milder reaction after the anterior chamber injection of the staphylococcus toxin than did the previous rabbits so treated.

Results

The results are shown in Figure 18. The control (Fig. 19) developed a mild iritis of approximately 24 hours duration after which the eyes rapidly cleared. The rabbits injected in the anterior chamber with both beef (Fig. 20) and pork ACTH (Fig. 21) showed exactly similar reactions. The only difference in the appearance of the eyes from those of the controls was the persisting unabsorbed

ACTH in the anterior chamber. The rabbits injected with cortisone again behaved quite differently. Two rabbits showed a slight traumatic reaction when examined eight hours afterwards, which accounts for the slight rise in the

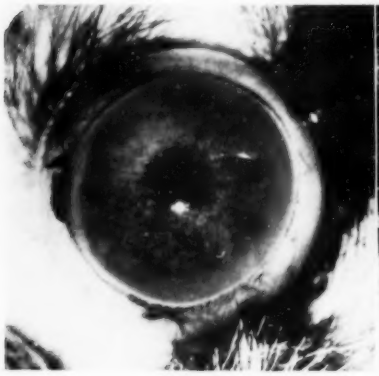

FIG. 19

FIG. 20

FIG. 19. Untreated control. Anterior chamber injection 0.1 cc. 1:100 staphylococcus toxin.
FIG. 20. Simultaneous injection of beef ACTH and 0.1 cc. 1:100 staphylococcus toxin in anterior chamber. Residual ACTH seen. No block.

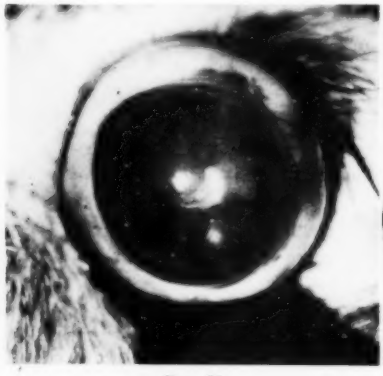

FIG. 21

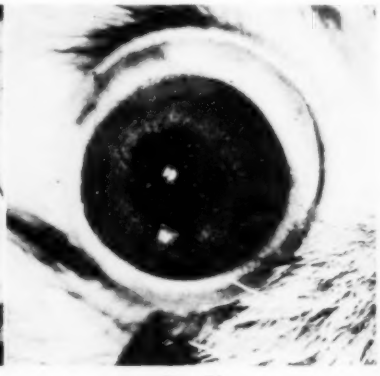

FIG. 22

FIG. 21. Simultaneous injection of pork ACTH and 0.1 cc. staphylococcus toxin in anterior chamber. Slight residual ACTH seen. No block.
FIG. 22. Simultaneous injection of cortisone and 0.1 cc. staphylococcus toxin in anterior chamber. All cortisone absorbed. Complete block.

cortisone curve in Figure 18. In 24 hours this subsided, and the eyes of all the cortisone rabbits were white and free of any evidence of inflammation (Fig 22). They remained so during the following two days, by which time the reaction in the eyes of the control and ACTH rabbits had also subsided.

COMMENT

The indication of these experiments appears clear. Primarily, while it is clear that parenteral pre-treatment with cortisone and pork ACTH results in a blocking of the inflammatory reaction produced by irritants, this blocking action is relative and largely on a quantitative basis. When strong jequirity infusions are injected and the insult to the eye is great, neither cortisone nor ACTH have any pronounced blocking effect. When weaker jequirity infusions are injected and the insult is so reduced, pre-treatment with cortisone and pork ACTH readily controlled the inflammation during the period of administration. When treatment with these agents was terminated, the inflammation slowly recurred. The irritant was still present and active in the eye as the protective action of cortisone and pork ACTH slowly faded. With a minimal insult, such as produced by the anterior chamber injection of 0.1 cc. of a 1:100 staphylococcus toxin, pre-treatment with cortisone and pork ACTH gave sufficient protection to block the inflammation during the short life of the irritant.

A notable observation was the comparative failure of beef ACTH given parenterally to inhibit the inflammation produced in a rabbit's eye by the anterior chamber injection of either jequirity or staphlococcus toxin. The preparation of beef ACTH used was one of established potency when used clinically. Apparently however, it failed to stimulate the adrenal cortex of the rabbit. All that can be said from these experiments is that in the rabbit parenterally injected pork ACTH was the equal of cortisone in blocking the inflammation produced by such irritants as jequirity and staphylococcus toxin, while beef ACTH was decidedly less active.

The third finding is about what would be expected. While cortisone injected in the anterior chamber has a blocking action, ACTH has none when similarly injected. It has been abundantly shown that cortisone applied topically has a local action, independent of any systemic response. This is remarkable when the relative insolubility of the acetate is considered. It is also astounding that when cortisone is injected in the anterior chamber, all trace of the substance disappears within 12–18 hours, while when ACTH is similarly instilled, it remains as a flocculent material in the lower part of the anterior chamber for several days. Cortisone quite evidently undergoes some change in the aqueous or body tissues which make it readily soluble and easily absorbed. Just what produces the change in solubility is not yet known. Since ACTH acts through stimulation of the host's adrenal cortex no local effect would be expected after topical application. This is exactly what was found in these experiments.

CONCLUSIONS

1. Both cortisone and pork ACTH given parenterally 3 days prior to ocular injection and one day thereafter, effectively block the inflammatory reaction

in the eye produced by the injection of weak jequirity infusion and 1:50 and 1:100 dilution of staphylococcus toxin.

2. This blocking action is relative and is either absent or only slightly evident when jequirity infusions sufficiently strong to produce tissue necrosis are injected in the eye.

3. Under the conditions of this experiment, the parenteral injection of the preparation of beef ACTH used was relatively ineffective in blocking the inflammation which follows the injection of either jequirity infusion or staphylococcus toxin in the anterior chamber of the rabbit's eye.

4. Cortisone injected in the anterior chamber synchronous with the injection of these irritants, has a local blocking action on the inflammatory response. Neither beef or pork ACTH has any such action applied topically.

The ACTH used in these experiments was provided by Armour and Co. through the courtesy of Drs. John R. Mote and Harley E. Kluxton, Jr.

STUDIES OF COMBINATIONS OF ANTIBIOTICS IN VITRO AND IN EXPERIMENTAL INFECTIONS IN MICE

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The clinical use of combinations of two or more antibacterial agents is being increasingly advocated, in the hopes of achieving various ends. Lehr and his associates (1), for instance, found that larger amounts of certain sulfonamides could be given without danger of kidney damage if the dose was made up of two or more different drugs. Others have proposed combining antibacterial agents in order to reduce the chances of developing resistant strains of bacteria. But the reason most frequently advanced for the use of combinations is to bring a wider range of microorganisms under the influence of chemotherapy than is possible with a single agent. Thus combinations are advocated for the treatment of mixed infections, or in cases where the etiological agent is unknown at the time when therapy is begun.

Several experimental studies have already been made (2–16) of the effects of combinations of antibiotics *in vitro* and *in vivo*, but in the main these have been limited to observations on two or three agents. Moreover evidence has been presented that shows that not all combinations are happy ones. It seemed therefore that it might be advantageous to make a thorough survey of this field of chemotherapy, and the present study was undertaken to determine, experimentally, which combinations of the five or six commonly used antibiotics are desirable and which should be avoided.

DEFINITIONS

As others have pointed out, the addition of one antibiotic to another can have one of four possible effects upon the latter. Its activity may be unchanged (no effect); it may be impaired (antagonism); it may be improved, but only to the extent that would be the case had an *equivalent* portion of the same antibiotic been added (additive effect); or, finally the antibacterial activity may be greater than that expected from simple addition. The word "synergism" is generally used to express this kind of interaction and will be employed in the present paper, in spite of the fact that at times the effect appeared to be one-sided.

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METHODS

Three methods of determining the effect of combinations of antibiotics on bacteria were employed. Two were tests *in vitro* and one was *in vivo*. One of the *in vitro* tests consisted of finding the minimal inhibitory concentration (m.i.c.) of the agents alone and in combinations, by the tube dilution technique. The other involved observations of the rate of multiplication of bacteria by means of interval colony counts. For the therapeutic work, infections, induced in mice with three strains of bacteria, were treated with the various combinations.

Six antibiotics were studied: penicillin G, aureomycin, chloromycetin (chloramphenicol), terramycin, streptomycin, and polymyxin D.*

In vitro tests

The basic medium was Difco Heart Infusion Broth, with 0.075 per cent dextrose added. It was used for making the antibiotic solutions as well as for the bacterial suspensions, and as the diluent. In tests with streptococci and staphylococci, it was enriched by the addition of 4 per cent defibrinated rabbit's blood to the final dilution of the bacterial suspension.

A. End point method. The general plan of this part of the study was to test the action of combinations of various multiples of minimal inhibitory concentrations (m.i.c.) of two antibiotics. The first step was to determine roughly, by serial two-fold dilutions, the m.i.c. of each agent alone. Next a finer end point was obtained by the use of more gradual dilutions. The final tests were set up as follows:

Two solutions of each antibiotic were prepared: One, used as Agent 1, contained 2.8 m.i.c./ml and the other, used as Agent 2, contained 4 m.i.c./ml. On a given day each antibiotic was used in both capacities.

In the first four tubes in a row of sixteen Wassermann tubes was placed 0.28 m.i.c./ml (final concentration) of Agent 1; in tubes 5 to 8 inclusive was placed 0.56 m.i.c.; in tubes 9 to 12 inclusive, 0.98 m.i.c.; and in the last four tubes, 1.40 m.i.c. As many such rows were prepared as there were agents to be tested. To the second, third, and fourth tubes in each of the four groups in a single row was added 0.2, 0.4, and 0.6 m.i.c./ml, respectively, of Agent 2. The contents of the tubes were brought up to a volume of 1.5 ml by the addition of plain broth. They were inoculated with 0.5 ml of bacterial suspension, thus making the final volume in each tube 2 ml. See Protocol 1.

The procedure was to pipette first the broth diluent into the tubes, then Agent 1. After an interval of 1½ to 2 hours Agent 2 was added, and the tubes were inoculated. They were incubated at 37°C for 17 or 18 hours, examined for growth, as shown by turbidity or haemolysis, and discarded.

In the first row of every set the same antibiotic was used as Agents 1 and 2. This row, therefore, was the control for the single antibiotic, and the end point in it gave the m.i.c. of that antibiotic under the actual conditions of the tests. Tube 9 (see Protocol 1) contained the closest approximation to the estimated m.i.c. of Agent 1. If the estimate was correct this tube showed no growth, whereas tubes 1 and 5 were turbid. With Agent 2 the same as Agent 1 tube 7 also contained almost 1 m.i.c., while tubes 8 and 10 and up contained more than 1 m.i.c. All these tubes, therefore, showed no growth. In the actual course of events, the end point for the single agent was seldom where it was expected to be. This necessitated a recalculation of the number of inhibitory concentrations per ml in both solutions of the agent. If, for instance, the end point for the single agent was in tube 6, the m.i.c. per ml was greater than

* The authors are indebted to the Lederle Laboratories, Inc., Parke Davis and Company, Charles Pfizer and Company, E. R. Squibb and Sons, and the American Cyanamide Company for generous supplies of these antibiotics.

had been expected, and the true proportion in each tube could be arrived at by multiplying the estimated value by 98/76, or 1.29. As Agent 1, therefore, the four concentrations of the antibiotic would be approximately 0.36, 0.72, 1.26, and 1.80 m.i.c./ml (instead of .28, .56, etc.) and the three concentrations as Agent 2 would be 0.26, 0.52, and 0.78 m.i.c./ml (instead of .2, .4, and .6).

Interpretation. The effect of a combination was judged according to whether the end point of the titration (i.e. the lowest concentration preventing growth) occurred in the presence of 1, more than 1, or less than 1 total m.i.c. (Protocol 1, line 8.) The effect was recorded as additive when the end point lay between 0.9 and 1.2 m.i.c. Synergism was denoted by an end point below 0.9 m.i.c. End points of 1.2 or above represented either no effect or antagonism. A diagnosis of antagonism was made when growth was present in a tube containing 1 or more m.i.c. of *one* of the two agents. Note that it appears possible, here, to demonstrate synergism for one of the components of a combination and not for the other. In reality, there is no discrepancy. As Price and his associates (5), Lankford and Lacy (6) and Bigger (10) have shown,

PROTOCOL 1

LINE	MATERIAL	CONC.	TUBE NO.															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
			ml															
1	Broth		1.3	1.2	1.1	1.0	1.1	1.0	0.9	0.8	0.8	0.7	0.6	0.5	0.5	0.4	0.3	0.2
2	Agent 1	2.8 m.i.c./ml	.2	.2	.2	.2	.4	.4	.4	.4	.7	.7	.7	.7	1.	1.	1.	1.
3	Agent 2	4.0 m.i.c./ml	0	.1	.2	.3	0	.1	.2	.3	0	.1	.2	.3	0	.1	.2	.3
4	Inoculum		0.5 ml in all tubes															
5	Total volume		2.0 ml in all tubes															
			Final concentration: m.i.c./ml															
6	Agent 1		.28	.28	.28	.28	.56	.56	.56	.56	.98	.98	.98	.98	1.4	1.4	1.4	1.4
7	Agent 2		0	.2	.4	.6	0	.2	.4	.6	0	.2	.4	.6	0	.2	.4	.6
8	Combined total		.28	.48	.68	.88	.56	.76	.96	1.16	.98	1.18	1.38	1.58	1.4	1.6	1.8	2.0

two agents may be synergistic when combined in one proportion and ineffective or even antagonistic when combined in other proportions.

Eighteen strains of bacteria were submitted to this test—six strains of *Staphylococcus aureus*, six of streptococci, and six of gram-negative bacilli. The streptococci included one strain of pneumococcus type I, a Group A haemolytic streptococcus, three strains of *Streptococcus faecalis*, and one member of the viridans group. Among the gram-negative bacilli were two strains of *E. coli*, and one each of *E. communior*, *A. aerogenes*, *K. pneumoniae* (type A), and an organism identified as *Salmonella typhimurium*. All of the organisms had been associated with human infections, and most of them had been isolated from patients quite recently.

The cultures, inoculated at 4 P.M., were incubated overnight and placed in the refrigerator until just before they were to be used. The haemolytic streptococcus and the pneumococcus were diluted 1:5000, and the other organisms 1:10 000.

B. Growth studies. Test tubes containing 9.5 ml of broth solutions of the antibiotics, alone and in combination, were inoculated with 0.5 ml of 1:1000 or 1:2000 blood broth dilutions of the test cultures. The cultures were eighteen hours old. Blood agar plates were poured, with suitable dilutions, after 1, 3, 5, 24, 48, and 72 or 96 hours of incubation at 37°C, and the

resulting colonies were counted. Two or three concentrations of each antibiotic were used. At first, tests were made with only 1 and 4 m.i.c./ml and these were combined in various ways. Later, the tests were made with 1, 2, and 4 m.i.c./ml of each agent alone, and with combinations of 1 m.i.c. of one agent, with 1 m.i.c. of the other, and of 4 m.i.c. with 4 m.i.c. See Protocol 2. Three antibiotics—sixteen cultures, including the control—proved the most satisfactory number to run on a given day. The organisms tried in this manner were the three used to produce the experimental infections: *K. pneumoniae*, strain KpnAD; the haemolytic streptococcus, C203, and the pneumococcus, SV1.

Interpretation. A comparison was made of the population-time curves derived by plotting (log) colony counts vs time of plating. When the curve for the smaller of the two combinations coincided with the curve for the least concentration of one of the antibiotics the interpretation was "no effect" for that antibiotic. When it approached the curve for the double portion an additive effect was indicated. Between these two there was a zone where the effect was termed indefinite. Curves falling outside the limits set by the single and the double portions of the agents connoted antagonism or synergism as the case might be. Since the agents were mixed in only two or three proportions the results obtained by this method were less

PROTOCOL 2

AGENT	CONC.	A			B			C			A + B		A + C		B + C		CONTROL
		Tube No.															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		ml															
A	10 m.i.c./ml	1	0	0							1	0	1	0			
	40 m.i.c./ml		.5	1							0	1	0	1			
B	10 m.i.c./ml	0			1	0	0				1	0			1	0	
	40 m.i.c./ml				0	.5	1				0	1			0	1	
C	10 m.i.c./ml							1	0	0			1	0	1	0	
	40 m.i.c./ml							0	.5	1			0	1	0	1	
Broth		8.5	9	8.5	8.5	9	8.5	8.5	9	8.5	7.5	7.5	7.5	7.5	7.5	7.5	9.5
Inoculum		0.5 ml in all tubes															

definite than those obtained by the end point technique. Synergism had to be a mutual effect, and, if a combination appeared to be much better with respect to one of the components than the other, its activity usually could be attributed to the stronger agent. For example, the combination of aureomycin and penicillin, which was less effective than penicillin alone but more effective than a double portion of aureomycin, was considered not to be synergistic with respect to aureomycin but to owe its activity to its penicillin content.

Therapeutic tests

CF_1 mice weighing 18 to 20 grams were used. They were infected by the intraperitoneal injection of broth suspensions of the bacteria. For the pneumococcal infection, the dose of organisms was ½ ml of a 1:50,000 dilution. This inoculum contained approximately 5000 diplococci, by colony count, and represented approximately 5000 minimal lethal doses. Smaller doses of the streptococcus and klebsiella were employed, that is, 12 to 300 MLD of the former and 100 to 500 of the latter. The organisms were passed through mice the day before a test, and their virulence was estimated each time from the mortality in groups of two to five mice inoculated with ten-fold dilutions of the culture.

Treatments were given by the subcutaneous route, immediately after the injection of the bacteria and again at 5½ and 23 hours. The dose of antibiotic was contained in 0.2 ml of water, except in the case of chloromycetin. The limit of solubility of this agent, in water, is about 4 mg per ml, and, when doses greater than 40 mg per kilo were wanted, larger volumes were injected. This measure was thought to be preferable to using alcoholic solutions, as had been the practice hitherto.

Efforts were made through preliminary testing to determine the amounts of the single antibiotics which would protect 10 to 20 per cent of the mice. This amount, and its double, were used as controls of efficacy in the experiments. The combinations were made by mixing equal parts of the lesser doses of the two antibiotics. They were mixed just before they were to be used, at each treatment period, according to Protocol 3.

For every combination tested there were four sets of treated controls, that is, mice treated with single and double portions of the two antibiotics making up the combination. As a rule, combinations of five antibiotics were tested at one time (polymyxin was not included in the therapeutic study), but occasionally smaller experiments, with only two or three combinations, were run. Ten or fifteen mice were used for each dose or combination in every experiment.

PROTOCOL 3

AGENT	CONC.	TUBE NO.				
		1	2	3	4	5
	mg/ml	*ml*				
Penicillin	0.2	1.0	2.0	0	0	1.0
Aureomycin	1.0	0	0	1.0	2.0	1.0
Water		1.0	0	1.0	0	0
		dose for 20 gram mouse				
		mg/kilo—in 0.2 ml				
Penicillin		1.0	2.0	0	0	1.0
Aureomycin		0	0	5.0	10	5.0

Interpretation. Survival time as well as per cent survival was considered in analysing the results of the therapeutic trials. Curves so derived are analogous to those of the growth study and carry the same interpretation.

RESULTS

In vitro. End point method

In Table I is presented a sample of the results obtained with combinations in tests run by the end point method, *in vitro*, and in Table II all the results obtained by this technique are summarized. In the first place it is seen, in this second table, that the most frequent result was an additive effect. Of the total of 415 tests (18 organisms; 20 to 25 combinations) 44 per cent showed an additive effect, 30 per cent showed synergism, 18 per cent: "no effect," and in only 7 per cent was outright antagonism demonstrated.

Aureomycin was the agent most likely to have its activity increased by the addition of another, but terramycin and penicillin were the most effective adjuvants.

Of the 28 tests in which antagonism was observed, 23 involved streptomycin. Twenty-one occurred as the result of adding aureomycin, chloromycetin, or terramycin to streptomycin, the addition of chloromycetin being especially harmful. The effect was most pronounced against the gram-positive organisms. This difference between gram-positive and gram-negative bacteria is seen again

TABLE I

Effects of Combinations of Antibiotics in Vitro. Example of Results Obtained by the Tube Dilution Technique

(Staph. aureus—strain Porter)

COMBINATION	GROWTH		GROWTH		INTERPRETATION (Effect on first agent)
	+	−	+	−	
	No. of m.i.c. of each agent		Total m.i.c. in combination		
			*	†	
Terra + peni	.24 + .34	.24 + .51	.58	.75	Synergistic
Peni + terra	.48 + .17	.48 + .34	.65	.82	Synergistic
Terra + aureo	.83 + .11	.83 + .22	.94	1.05	Additive
Aureo + terra	.53 + .17	.53 + .34	.70	.87	Synergistic
Terra + chloro	.48 + .11	.48 + .22	.59	.70	Synergistic
Chloro + terra	.49 + .17	.49 + .34	.66	.83	Synergistic
Terra + strepto	.83 + .71	.83 + 1.06	1.54	1.89	No Effect
Strepto + terra	1.00 + .51	1.75 + .17	1.51	1.92	Antagonistic

* Highest concentration permitting growth.

† Lowest concentration preventing growth.

Abbreviations used in this and the succeeding tables: Aureomycin: Aureo, Aur, A; Polymyxin D: Poly, Pol; Chloromycetin: Chlor, Chl, C; Terramycin: Terra, Ter, T; Penicillin: Peni, Pen, P; Streptomycin: Strepto, Str, S; Additive: Add; Synergistic: Syn; Antagonism: Ant; No Effect: n.e.

in the responses to the addition of streptomycin to chloromycetin. With the cocci the best result of this combination was "no effect."

In contrast to the foregoing, the combination of terramycin with chloromycetin was synergistic in 22 of the 26 tests. No antagonism was noted and "no effect" was observed only four times. The six strains of staphylococci responded least favorably to this combination.

In vitro. Growth studies

Multiplication-time curves for the streptococcus, C203, in the presence of chloromycetin or terramycin and combinations of these are shown in Figure 1,

TABLE II

Effects of Combinations of Antibiotics in Vitro

Summary of Results Obtained with 18 Strains by Tube Dilution Technique

COMBINATION	STAPHYLOCOCCI 6 strains				STREPTOCOCCI 6 strains				GRAM—BACILLI 6 strains				ALL STRAINS			
	Effect—number of times noted															
	Syn.	Add.	N.E.	Ant.	Syn.	Add.	N.E.	Ant.	Syn.	Add.	N.E.	Ant.	Syn.	Add.	N.E.	Ant.
Peni + aur	2	4	0	0	2	4	0	0	1	3	1	0	5	11	1	0
chl	1	3	2	0	1	3	2	0	0	1	3	1	2	7	7	1
ter	4	2	0	0	1	3	2	0	2	1	2	0	7	6	4	0
str	2	4	0	0	3	2	1	0	2	2	1	0	7	8	2	0
pol	not done				not done				1	1	2	1	1	1	2	1
Aureo + pen	2	4	0	0	3	3	0	0	4	2	0	0	9	9	0	0
chl	1	2	3	0	2	4	0	0	4	2	0	0	7	8	3	0
ter	4	2	0	0	2	4	0	0	2	3	1	0	8	9	1	0
str	2	4	0	0	4	2	0	0	4	1	1	0	10	7	1	0
pol	not done				not done				3	2	0	1	3	2	0	1
Chlor + pen	1	4	1	0	0	4	2	0	1	3	2	0	2	11	5	0
aur	1	5	0	0	3	3	0	0	3	3	0	0	7	11	0	0
ter	2	4	0	0	5	1	0	0	5	1	0	0	12	6	0	0
str	0	0	4	2	0	0	6	0	1	3	2	0	1	3	12	2
pol	not done				not done				1	3	1	1	1	3	1	1
Terra + pen	3	2	1	0	1	4	1	0	3	2	1	0	7	8	3	0
aur	1	3	2	0	1	5	0	0	1	4	1	0	3	12	3	0
chl	1	1	4	0	4	2	0	0	5	1	0	0	10	4	4	0
str	0	1	5	0	1	4	1	0	2	3	1	0	3	8	7	0
pol	not done				not done				0	5	1	0	0	5	1	0
Strep + pen	3	2	1	0	0	3	3	0	4	2	0	0	7	7	4	0
aur	0	1	2	3	1	4	0	1	2	3	1	0	3	8	3	4
chl	0	0	0	6	0	0	1	5	0	3	1	2	0	3	2	13
ter	0	1	2	3	0	5	0	1	3	3	0	0	3	9	2	4
pol	not done				not done				1	4	1	0	1	4	1	0
Poly + pen									2	1	2	1	2	1	2	1
aur									2	4	0	0	2	4	0	0
chl									1	2	3	0	1	2	3	0
ter									0	5	1	0	0	5	1	0
str									1	3	2	0	1	3	2	0
Total													125	185	77	28
%													30	44½	18½	7

to illustrate the results obtained by this method. The combination of one m.i.c. of chloromycetin with one m.i.c. of terramycin was more inhibitory than two m.i.c. of either agent and is, therefore, considered to be synergistic. The combination of four m.i.c. of each was only slightly more effective than four m.i.c.

of chloromycetin and is termed additive for this agent. The curve for this combination was almost indistinguishable from that for four m.i.c. of terramycin for the first five hours of the test, but as time went on the difference between the two became more and more pronounced. The effect of adding four m.i.c. of chloromycetin to four m.i.c. of terramycin is, therefore, termed "no effect" followed by an additive effect.

Mixed results of this latter type were observed quite often. They are designated n.e./syn or ant/n.e., etc., in Table III, in which are summarized the results of the growth studies.

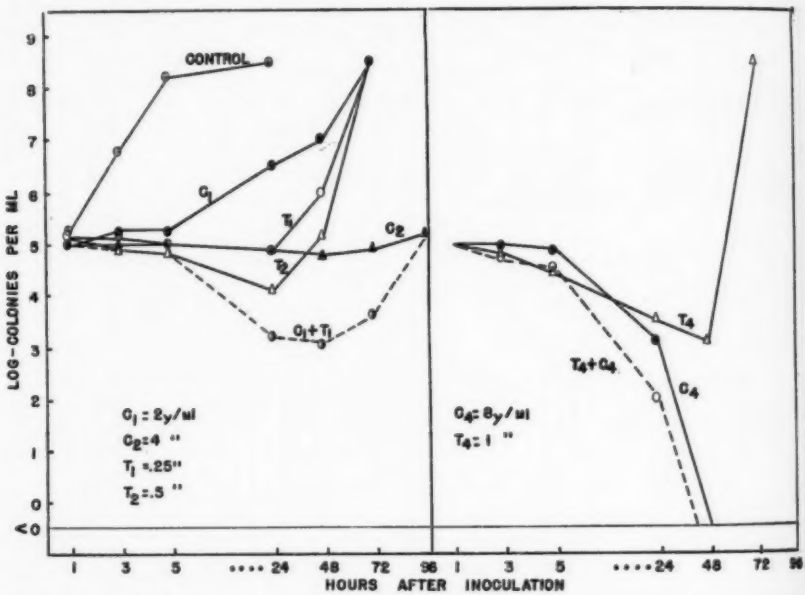

FIG. 1. Multiplication-time curves for β streptococcus, C203, in presence of chloromycetin and terramycin. $C_1 + T_1$: synergistic. $C_4 + T_4$: additive for C; no effect, then additive for T.

Because of the fact that the agents were combined in more than one proportion, and with varying effect, it is difficult at first to discern any pattern in the material presented in Table III. However, it is clear that the association of penicillin with streptomycin was synergistic. Chloromycetin and terramycin also were more effective in combination than when used separately. Penicillin's activity was diminished by the addition of aureomycin or chloromycetin but, with the lower concentrations, this harmful effect was transient. It is interesting to note that in the growth studies antagonism was demonstrated only in the presence of relatively high concentrations of the agents.

Although greater antagonism between penicillin and aureomycin, and less antagonism between chloromycetin and streptomycin were brought out in the

TABLE III

Effects of Combinations of Antibiotics in Vitro
Summary of Results Obtained from Growth Studies of 3 Strains

COMBINATION	SV1		C203				FRIEDLAENDER A	
	Number of m.i.c. combined							
	1 + 1	4 + 4	1 + 1	4 + 4	1 + 4	4 + 1	1 + 1	4 + 4
	Effect on 1st agent*							
					(P_1+X_4)	(P_4+X_1)		
Peni + aureo	n.e./Syn	Ant	n.e./Add	Ant	Ant/=A_4	Ant	n.e./Syn	Ant
Aureo + peni	n.e./Syn	Ant	=P_1	=P_4	n.e./Add	=P_4	n.e./Syn	=P_4
Peni + chloro	Add	Ant			Ant/=C_4	Ant	Add	Ant
Chloro + peni	Add	Ant			Add	=P_4	Add	=P_4
Peni + strepto	Syn	Syn	Syn	=S_4	Syn	Syn	Syn	Syn
Strepto + peni	Syn	Syn	Syn	Add	Syn	Syn	Syn	Syn
Peni + terra					Syn	Ant		
Terra + peni					Syn	=P_4		
Aureo + chloro	Syn	=C_4	Syn	Add			Syn	Add/Syn
Chloro + aureo	Syn	n.e.	Syn	Add			Syn	Add/Syn
Aureo + strepto	Syn	Syn	Syn	=S_4			Syn	=S_4
Strepto + aureo	Syn	Syn	Syn	n.e.			Syn	Ant
Aureo + terra			n.e./Add	n.e./Add				
Terra + aureo			n.e./Add	n.e./Add				
					(C_1+S_4)	(C_4+S_1)		
Chloro + strepto	Add	=S_4	n.e./Syn	=S_4	=S_4	n.e.	n.e./Syn	=S_4
Strepto + chloro	Add	n.e.	n.e./Syn	Ant/n.e.	Ant/n.e.	=C_4	=C_4/Syn	n.e.
Chloro + terra	Syn	Syn	Syn	Add			Add	Syn
Terra + chloro	Syn	Syn	Syn	n.e./Add			Add	Syn
Poly D + peni							Syn	n.e.
Peni + poly							Syn	=$Poly_4$
Poly D + aureo							Syn	Ant
Aureo + poly							Syn	=$Poly_4$
Poly D + chloro							Syn	n.e.
Chloro + poly							Syn	=$Poly_4$
Poly D + strepto							Indef	Add
Strepto + poly							Indef	Add

* n.e. = no effect; n.e./Syn = no effect at first, synergistic later.
Indef = better than no effect but not equal to additive.
=P_1 etc. means effect due to penicillin, etc., content.

growth studies than by the end point method, most of the results obtained by the two methods are similar. Such differences as are noted can be accounted

for by differences in reading time and in the relative concentrations of the antibiotics. With the end point technique, only the 18 hour effect was registered and the combinations were in the smallest possible proportions.

The effect of adding the second agent after a brief exposure to the first was next investigated. Penicillin is more active against rapidly dividing cells and it has been suggested (17) that the antagonism toward it, demonstrated by certain other antibacterial agents, might be caused by their inhibition of bacterial multiplication. The present experiments were prompted by some remarks of Doctor Ernest Jawetz of the University of California. Dr. Jawetz said

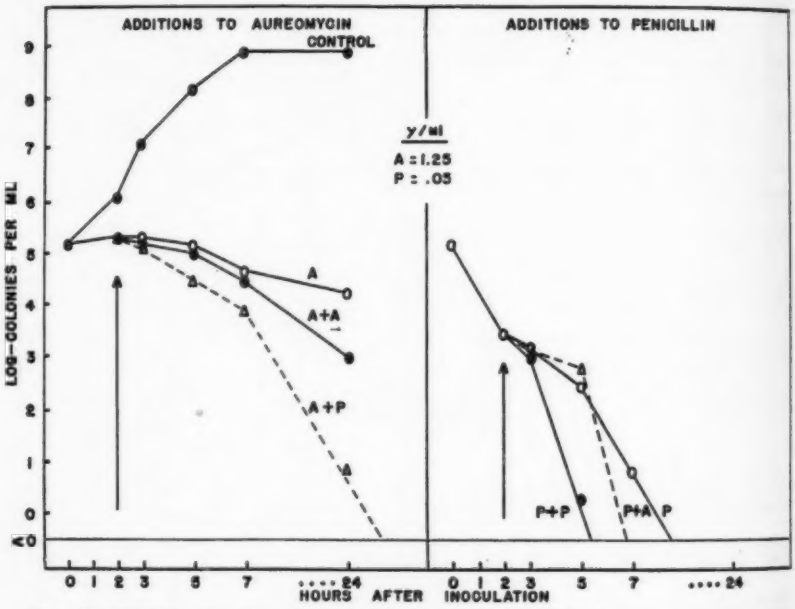

FIG. 2. Multiplication-time curves for β streptococcus C203, in presence of penicillin and aureomycin. Second agent added 2 hr. after inoculation. As indicated by arrow.

that he no longer believed that the "resting cell" theory provided the entire explanation of antibiotic antagonism because he had found that the addition of penicillin, to cultures already containing penicillin in bacteriostatic amounts, did not result in antagonism.

In the experiments illustrated in Figures 2 and 3 penicillin, aureomycin, and streptomycin were combined in various sequences. The cultures, containing one antibiotic and inoculated with the streptococcus, C203, were prepared as for the usual growth study, but in triplicate. After two hours at 37° C additions of antibiotic were made to two of the cultures and of plain broth to the third.

Plates were poured just before the addition and at 1, 3, 5, and 22 hours thereafter. In the first experiment, 4 m.i.c. of penicillin or 4 m.i.c. of aureomycin were added after two hours to cultures containing penicillin and to cultures containing aureomycin, with the results shown in Figure 2.

Doubling the amount of aureomycin, at 2 hours, caused a small increase in the rate of decline of the bacterial count. Addition of penicillin to aureomycin at this time had a greater effect. Addition of either penicillin or aureomycin to the penicillin culture was without apparent action for one hour, but in another two hours the culture containing the double quantity of penicillin

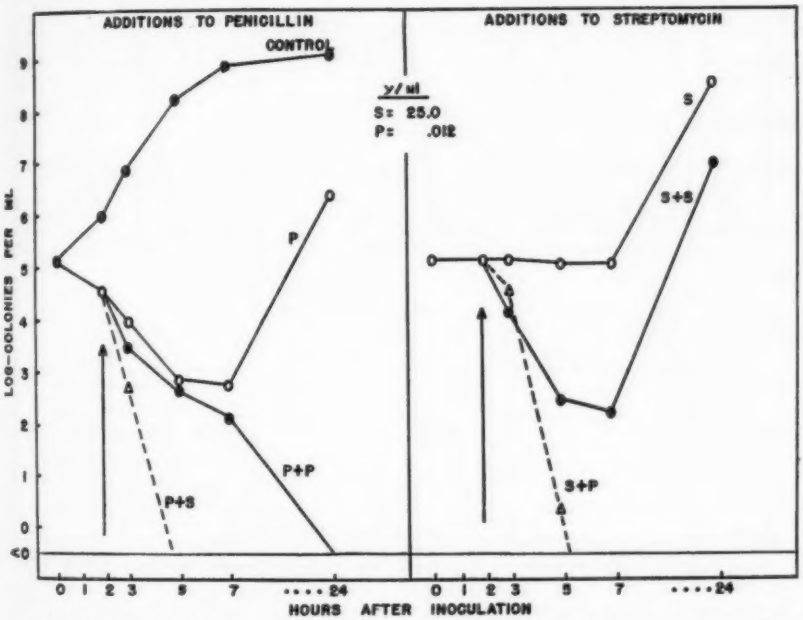

FIG. 3. Multiplication-time curves for β streptococcus, C203, in presence of penicillin and streptomycin. Second agent added 2 hr. after inoculation. As indicated by arrow.

was sterile. The culture to which aureomycin had been added was almost indistinguishable from the one containing the single portion of penicillin. However, the slightly higher count of the former at 5 hours is perhaps significant since it was observed on repetition of the test. At any rate, it is clear that penicillin exercises an antibacterial effect even when added to cultures that are in a "bacteriostatic" condition.

This observation was confirmed by the results obtained by combining penicillin and streptomycin, as shown in Figure 3. The addition, after two hours, of either agent to either agent resulted in an immediate acceleration of the de-

cline of the bacterial population. Synergism was demonstrated by the faster rate for the heterologous than for the homologous additions.

Therapeutic studies

Difficulties were encountered in the performance of the tests *in vivo*. One lay in variations in the pathogenicity of the microorganisms from one experiment to the next. This was particularly troublesome in the studies with the

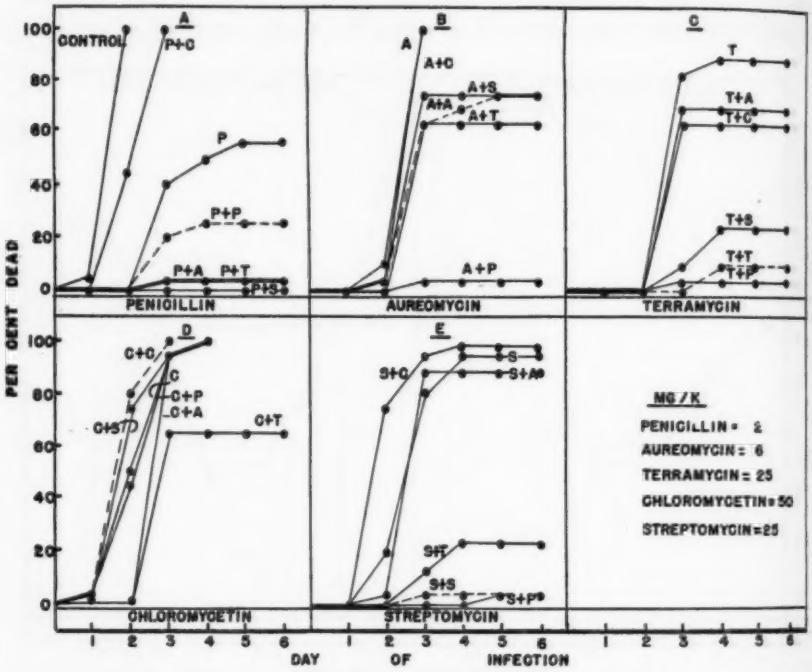

FIG. 4. Mortality-time curves. Pneumococcus SVI. Mice treated with combinations of 5 antibiotics. Pooled results of 4 experiments. Each group of curves shows the effects of combinations relative to *one* of the antibiotics.

haemolytic streptococcus, C203. Another, only partly related to the first, was inconsistency in the efficacy of the agents when used separately. A third difficulty was presented by differences in the slopes of the dosage survival curves of the various agents. For example, doubling the dose of Agent A raised the survival rate from 20 per cent to 100 per cent, but doubling Agent B raised it only from 20 to 30 per cent. Chloromycetin presented a special problem in this regard. Although it behaved normally in the Friedlaender's infection, in the coccal infections increasing the dosage led either to no improvement or to a

decrease in the survival rate. These difficulties interfered with the original plan of determining the median protective doses of the agents for the three infections, and it was impossible to make statistical analyses of the data. Hence only the most general conclusions will be drawn from the observations of the therapeutic activity of the combinations.

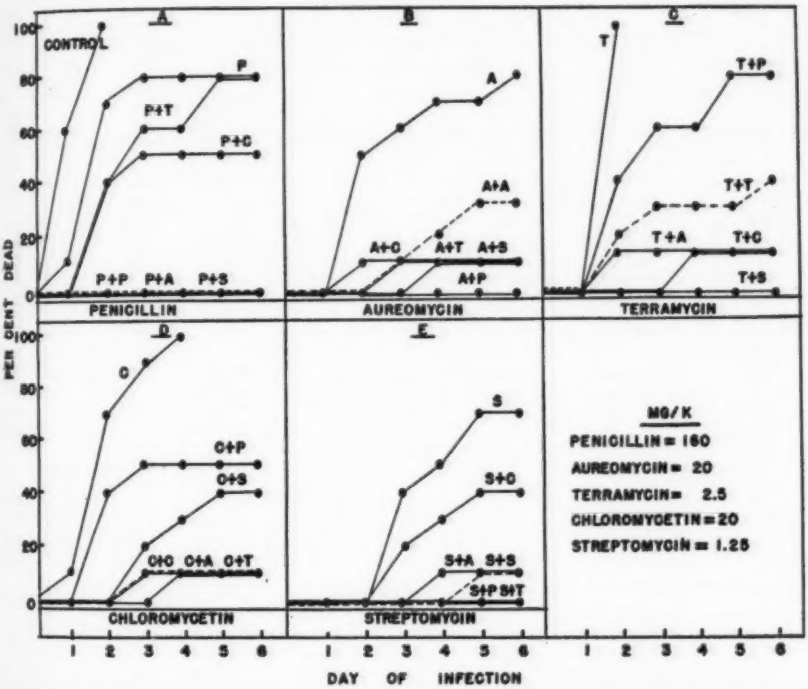

FIG. 5. Mortality-time curves. *K. Pneumoniae*, Kpn AD. mice treated with combinations of 5 antibiotics. Results of exp. 2. Each group of curves shows the effects relative to *one* of the antibiotics.

Four tests of the effects of combinations of antibiotics on the pneumococcal infection were made with results that were so much alike that it was possible to pool them. The combined mortality curves are shown in Figure 4.

The Friedlaender's infection was studied twice. The first time the doses of penicillin, aureomycin and chloromycetin were too small and that of streptomycin too large, so that anything added to the first three appeared to have a synergistic effect, and additions to streptomycin were at best additive. Doubling the dose in the one instance and halving it in the other brought the results illustrated in Figure 5. There were no unaccountable discrepancies between the two tests.

On the other hand, there was no consistency in the results of the four tests with the haemolytic streptococcus, and they have therefore been tabulated separately in Table IV.

TABLE IV

Effect of Therapy with Combinations of Antibiotics on Experimental Streptococcal Infections in Mice (Strain C203)

EXP. 1			EXP. 2			EXP. 3			EXP. 4		
Bact. count.....200 MLD..... 12			1000 80			500 300			800 90		
Agent & dose*	Died %	Interpretation†	Agent & dose	Died %	Interpretation	Agent & dose	Died %	Interpretation	Agent & dose	Died %	Interpretation
P .1	60		P .1	100		P .075	80		P .075	20	
+ P .1	30		+ P .1	67		+ P .075	25		+ P .075	0	
+ A .5	70	Ant	+ A .25	93	n.e.	+ A .5	100	Ant	+ A .5	53	Ant
+ T 1.0	20	Syn	+ T 1.0	73	Indef	+ T 1.0	80	n.e.	+ T 1.0	46	Ant
+ C 40	20	Syn	+ C 20	93	n.e.	+ C 20	80	n.e.	+ C 20	86	Ant
+ S 25	20	Add	+ S 20	80	Indef	+ S 30	30	Add	+ S 30	0	Syn
A .5	50		A .25	93		A .5	90		A .5	70	
+ A .5	20		+ A .25	100		+ A .5	70		+ A .5	40	
+ P .1	70	Ant	+ P .1	93	n.e.	+ P .075	100	Ant	+ P .075	53	= P
+ T 1.0	10	Syn	+ T 1.0	80	= T	+ T 1.0	40	Syn	+ T 1.0	46	Add
+ C 40	20	Add	+ C 20	73	Syn	+ C 20	50	Syn	+ C 20	66	n.e.
+ S 25	50	n.e.	+ S 20	100	n.e.	+ S 30	80	Indef	+ S 30	53	Indef
T 1.0	50		T 1.0	80		T 1.0	70		T 1.0	70	
+ T 1.0	40		+ T 1.0	43		+ T 1.0	80		+ T 1.0	26	
+ P .1	20	Syn	+ P .1	73	Indef	+ P .075	80	n.e.	+ P .075	46	= P
+ A .5	10	Syn	+ A .25	80	n.e.	+ A .5	40	Syn	+ A .5	46	Indef
+ C 40	0	Syn	+ C 20	73	Indef	+ C 20	70	n.e.	+ C 20	33	Add
+ S 25	40	Add	+ S 20	73	n.e.	+ S 30	60	Add	+ S 30	73	n.e.
C 40	0		C 20	86		C 20	60		C 20	66	
+ C 40	30		+ C 20	86		+ C 20	90		+ C 20	70	
+ P .1	20	Syn?	+ P .1	93	Ant	+ P .075	80	n.e.?	+ P .075	86	Ant
+ A .5	20	Add	+ A .25	73	Syn	+ A .5	50	Syn	+ A .5	66	n.e.
+ T 1.0	0	Syn?	+ T 1.0	73	Add	+ T 1.0	70	n.e.	+ T 1.0	33	Syn?
+ S 25	40	Ant	+ S 20	93	Ant	+ S 30	90	Ant?	+ S 30	80	Ant
S 25	60		S 20	100		S 30	100		S 30	100	
+ S 25	0		+ S 20	100		+ S 30	20		+ S 30	53	
+ P .1	20	Add	+ P .1	80	Add	+ P .075	0	Add	+ P .075	0	Syn
+ A .5	50	n.e.	+ A .25	100	n.e.	+ A .5	80	Indef	+ A .5	53	Add
+ T 1.0	40	Indef	+ T 1.0	73	= T	+ T 1.0	60	Indef	+ T 1.0	73	Indef
+ C 40	40	Indef	+ C 20	93	n.e.	+ C 20	90	n.e.	+ C 20	80	Indef

* Dose in mgm/kgm; 3 doses given.

† Effect relative to first agent:

Indef (indefinite) = better than "no effect," not as good as additive.

= P: effect due to penicillin content.

Interpretations of the findings in the three infections have been set up for comparison in Table V. Combinations of penicillin and streptomycin showed synergism in all three infections, but in the streptococcal infection other effects also were noted. Combinations of chloromycetin and streptomycin were never so much as additive. Combinations of terramycin with aureomycin, chloromycetin, or streptomycin were for the most part additive or synergistic—

never antagonistic. Combinations of aureomycin and chloromycetin and of aureomycin and streptomycin produced intermediate effects (additive or "no effect") in the klebsiellar and pneumococcal infections. In the streptococcal infection the second combination was ineffective, whereas aureomycin and chloromycetin at times acted synergistically.

TABLE V

Summary of the Effects of Combinations of Antibiotics in the Therapy of Experimental Infections in Mice

COMBINATION	PNEUMOCOCCUS SV1 (4 EXP. POOLED)	β STREPTOCOCCUS C203 (4 EXP. SUMMARIZED)	K. PNEUMONIAE KPN AD (2ND EXP.)	ALL THREE INFECTIONS
	Effect relative to first agent			
Peni + aureo	Syn	3 Ant, 1 n.e	Syn	Variable
Aureo + peni	Syn	2 Ant, 2 n.e	Syn	Variable
Peni + chloro	Ant	1 Ant to 1 Syn	Indef	Variable
Chloro + peni	n.e	2 Ant to 1 Syn	Indef	Variable
Peni + terra	Syn	1 Ant to 1 Syn	Indef	Variable
Terra + peni	Syn	1 n.e to 1 Syn	Indef	Variable
Peni + strepto	Syn	1 Syn to 1 Indef	Syn	Additive or synergistic
Strepto + peni	Syn	1 Syn, 3 Add	Syn	Additive or synergistic
Aureo + chloro	n.e	2 Syn to 1 n.e	Add	Variable
Chloro + aureo	Add	2 Syn to 1 n.e	Add	Variable
Aureo + terra	Add	2 Syn to 1 Indef	Syn	Additive or synergistic
Terra + aureo	Indef	2 Syn to 1 n.e	Syn	Variable
Aureo + strepto	Add	2 n.e, 2 Indef	Add	Intermediate
Strepto + aureo	n.e	2 n.e to 1 Add	Add	Intermediate
Chloro + terra	Add	2 Syn to 1 n.e	Add	Variable
Terra + chloro	Indef	1 Syn to 1 n.e	Add	Variable
Chloro + strepto	n.e	4 Ant	Indef	Less than additive
Strepto + chloro	Ant	2 n.e, 2 Indef	Indef	Less than additive
Terra + strepto	Add	2 Add, 2 n.e	Syn	Variable
Strepto + terra	Add	4 Indef	Syn	Better than no effect

The effect of combinations of agents, other than streptomycin, with penicillin varied with the infection. Penicillin plus aureomycin was synergistic in the pneumococcal and Friedlaender's infections, mainly antagonistic in the streptococcal infection. Penicillin and terramycin acted synergistically in the pneumococcal infection, variably against the streptococcus, while the response in the *K. pneumoniae* infection was "indefinite." (That is, the survival rate

was better than that obtained with a single dose of either agent, but not as good as that obtained with double doses.) Combinations of penicillin and chloromycetin were for the most part less than additive. In the pneumococcal infection where chloromycetin is peculiarly ineffective, the addition of chloromycetin to penicillin abolished the latter's considerable therapeutic activity.

TABLE VI

Comparison of Results with Combinations of Antibiotics According to Technique and Bacterial Strain

COMBINATION	TECHNIQUE AND STRAIN											
	End point			Growth study			Mouse therapy			All three methods		
	SV1	C203	Fried	SV1	C203	Fried	SV1	C203	Fried	SV1	C203	Fried
P + A	M*	S	M	M & A	M & A	M & A	S	M & A	S	S, M, A	S, M, A	S, M, A
A + P	M	M	S	M & A	M	M	S	M & A	S	S, M, A	M & A	S & M
P + C	M	M	M	M & A	A	M & A	A	S & A	M	M & A	S, M, A	M & A
C + P	M	M	M	M & A	M	M	M	S & A	M	M & A	S, M, A	M
P + T	M	M	M		S & A		S	S & A	M	(S & M)	S, M, A	(M)
T + P	M	M	M		S & M		S	S & M	M	(S & M)	S & M	(M)
P + S	S	M	M	S	S & M	S	S	S & M	S	S	S & M	S & M
S + P	M	M	M	S	S & M	S	S	S & M	S	S & M	S & M	S & M
A + C	M	S	S	S & M	S & M	S & M	M	S & M	M	S & M	S & M	S & M
C + A	S	S	S	S & M	S & M	S & M	M	S & M	M	S & M	S & M	S & M
A + T	S	M	S		M		M	S & M	S	(S & M)	S & M	(S)
T + A	M	M	S		M		M	S & M	S	(M)	S & M	(S)
A + S	S	M	S	S	S & M	S & M	M	M	M	S & M	S & M	S & M
S + A	M	A	M	S	S & M	S & A	M	M	M	S & M	S, M, A	S, M, A
C + T	S	S	S	S	S & M	S & M	M	S & M	M	S & M	S & M	S & M
T + C	M	S	S	S	S & M	S & M	M	S & M	M	S & M	S & M	S & M
C + S	M	M	M	M	M	M	M	A	M	M	M & A	M
S + C	M	A	A	M	M & A	M	A	M	M	M & A	M & A	M & A
T + S	M	M	M				M	M	S	(M)	(M)	(S & M)
S + T	M	A	M				M	M	S	(M)	(M & A)	(S & M)

* Type of reaction; M = intermediate; S = synergism; A = antagonism.

Mice treated with the combination died as fast as those that received chloromycetin alone, and almost as fast as the controls.

Comparison of methods

In order to compare the different ways of measuring the effects of the combinations, the results obtained with the three strains of bacteria that were tested by all three methods are summarized in Table VI. To simplify tabulation, reactions intermediate between synergism and antagonism have been grouped together.

Agreement between the results obtained with a given combination, tested

by the three methods and against the three organisms, was only fair. All of the 20 combinations showed synergism or antagonism as well as an intermediate effect, and 7 showed all three effects. The extreme reactions, synergism and antagonism, were less frequently revealed by the end point method than by the growth studies or the therapeutic trials. Aside from this none of the techniques seemed especially inclined toward one of the reactions.

When the results are examined from the standpoint of a particular microorganism, agreement between the methods of testing appears better. It is least good in tests with the streptococcus, but this is explained, at least in part, by the fact that more growth studies were performed with this strain, and more therapeutic studies recorded, than with the other two. At the same time it should be borne in mind that the reason for the separate recording of the therapeutic studies with the streptococcus was that the results were so inconstant that they could not be pooled. With the pneumococcus the complete gamut of reactions—synergism, antagonism and intermediate effect—was noted only in the case of the aureomycin-penicillin combinations; with Friedlaender's bacillus it was seen only where aureomycin was added to penicillin or streptomycin; antagonism, in these instances was brought out in the growth studies but not in the other tests. With the streptococcus all three reactions were obtained with both chloromycetin-penicillin combinations, with terramycin added to penicillin and with aureomycin added to penicillin or streptomycin.

The data presented in Table VI also suggest that there is better accord between the effects of a combination upon three different microorganisms when studied by a single method than there is between its effects on one organism as observed by different methods. Effects upon the three strains were most homogeneous when they were determined by the end point method, least so when determined by therapeutic trials.

Finally, the data show that combinations of penicillin with aureomycin, chloromycetin or terramycin were the most variable in their effects. All such combinations displayed antagonism under at least one set of conditions, while synergism was frequently noted. It is curious that the end point method failed to reveal any antagonism in this group of combinations, yet showed it strongly in the case of additions to streptomycin. In Table II two instances of antagonism to penicillin, as shown by the end point technique, are recorded; one was from the addition of polymyxin and one from chloromycetin; both occurred with a strain of *E. coli* as the test organism.

DISCUSSION

Synergism of penicillin and streptomycin, first noted by Klein and Kimmelman (2) is recognized by all who have studied this combination of antibiotics. Aureomycin and chloromycetin are usually reported as antagonistic toward

penicillin (5, 6, 9, 11, 13, 16) but several authors have pointed out that the effect varies with the concentration of the agents (5, 6, 15) and Spicer (11) got different results with different bacteria. The effect of varying the concentration is shown in the growth studies illustrated in Table III of the present paper. With combinations of 1 m.i.c. of each agent the poorest result, "no effect," was only transient, the final effect being additive or synergistic. When higher concentrations were used antagonism was revealed in several pairs of antibiotics. Furthermore, when different concentrations were used for the two antibiotics, antagonism was most marked if the more effective agent was present in the higher concentration.

Combining aureomycin with chloromycetin or streptomycin has resulted in an additive or synergistic effect, according to most reports (5, 12, 14, 16). Gunnison, Coleman and Jawetz (13) found that terramycin had much the same effect as aureomycin on penicillin. Armstrong and Larner (16) observed synergism in combinations of terramycin with aureomycin, chloromycetin or streptomycin. Price et al. (5) found polymyxin B to be synergistic with other antibiotics *in vitro*, but antagonistic toward penicillin and streptomycin *in vivo*.

With these observations the results of the present study are, for the most part, in accord. They are at variance, however, with respect to the effects of combining chloromycetin with streptomycin. Here, it was found that chloromycetin interfered with streptomycin's activity for the pneumococcus, the haemolytic streptococcus and the strain of *K. pneumoniae* by at least one of the methods of testing. Antagonism was striking in tests with staphylococci and streptococci by the end point method. Others have reported an additive or even a synergistic relationship between these agents (5, 10, 11, 12, 16). The disagreement may be due in part to the use of different test organisms; Armstrong and Larner, Price and his associates and Werner and Knight (12) all used gram-negative bacilli, but Bigger's test organism was *Staphylococcus aureus*.

It would be foolish to labour this point in view of the variability of the findings within this one study. Moreover, none of the synergisms or antagonisms that were uncovered was of a high order. The greatest antagonism noted by the end point method—the method that gives the most accurate information on the magnitude of an effect—was between chloromycetin and streptomycin, and this was not impressive. Although in one test with a staphylococcus chloromycetin interfered with 2 m.i.c. of streptomycin, the average interference, in the presence of staphylococci and streptococci, was with 1.5 and 1.0 m.i.c., respectively. Synergism, though more prevalent than antagonism, was if anything less significant.

With regard to the cause of the phenomena of synergism and antagonism, Bigger pointed out that synergistic relationships between two antibacterial agents are readily understood, for if the agents affect the same metabolic proc-

ess of the bacterium, they might be expected to reinforce each other; while if they operate at different loci, the bacterium conceivably receives twice as many injuries and is doubly damaged.

The explanation of antagonism is harder to reach. Jawetz and his associates have ruled out the possibility of a chemical inactivation of penicillin by aureomycin and chloromycetin and, like Bigger, believe that the interaction has a physiological basis. Competition for the same substrate was considered improbable because of the dissimilar chemical structures of these antibiotics. In their published reports, Jawetz and his associates seem to favour the hypothesis that prevention of cell division by aureomycin, chloromycetin and terramycin is the source of their antagonism toward penicillin. The tenability of this view is weakened by the observation that penicillin acted promptly on bacteria even when added after they had been exposed for two hours to another antibiotic and seemed no longer to be multiplying (cf. Figures 2 and 3). On the other hand, when aureomycin was added, after two hours, to a culture containing penicillin there was a slight, but definite, slowing of the rate of decline of the bacterial population. The disturbance of the ratio between multiplying and dying cells, indicated by this finding, suggests that aureomycin had either interfered directly with the bactericidal action of penicillin or had stimulated cell division.

Jawetz and his associates in their series of papers have ably discussed the importance of antibiotic synergism and antagonism with relation to the treatment of patients. They are careful to note the danger of transforming experimental findings into clinical precepts, mentioning among other things, the differences that they observed under varying conditions of dosage and timing of therapy. It is, however, becoming increasingly difficult to carry out *controlled* comparisons of antibacterial agents in patients. Although there is some evidence that the combination of penicillin with streptomycin is more effective than either agent alone in the treatment of subacute bacterial endocarditis —especially of the type caused by enterococci (18)—the incidence of this infection is so low as to make valid comparisons almost impossible.

The results obtained in the present experimental study are equally inconclusive—but for another reason. Except in the case of penicillin-streptomycin and chloromycetin-streptomycin combinations, most of the effects of combining two antibiotics were so variable or so trivial as to preclude unqualified endorsement or condemnation of any pair.

SUMMARY

The antibacterial activity of two-fold combinations of penicillin, aureomycin, chloromycetin, terramycin and streptomycin, and occasionally of polymyxin D, were tested *in vitro* and *in vivo*.

In vitro, the minimal inhibitory concentrations of the combinations, and

their effects on multiplication of bacteria, were determined. Eighteen strains of bacteria were used.

In vivo, studies were made of the therapeutic effects of the combinations in infections induced in mice with one strain each of pneumococcus, haemolytic streptococcus and *Klebsiella pneumoniae*.

Effects of the combinations varied with the method of testing, the test organism and, most importantly, with the concentrations of the two components. The greater the concentration of the agents the less was the benefit derived from combining them.

Synergism was demonstrated more frequently than antagonism, but most of the reactions fell between these two extremes, the effects of the combinations being merely additive or no different from those of the agents when used separately.

Combinations which were predominantly synergistic were those of penicillin with streptomycin and of aureomycin or terramycin with chloromycetin.

Antagonism was exhibited between chloromycetin and penicillin or streptomycin, *in vivo*, and by one or the other of the methods, *in vitro*. Aureomycin, also, acted antagonistically toward penicillin in the growth studies and in the experimental streptococcal infection. In the pneumococcal and klebsiellar infections, however, these two agents were synergistic.

The authors are indebted to Dr. Jacques Monnier of the Faculté de Médecine, Université de Toulouse, and Fellow in Preventive Medicine, The Johns Hopkins University, 1950–1951, for many helpful suggestions and for stimulating discussions of this work while it was in progress.

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OBSERVATIONS ON THE POSSIBLE ROLE OF NUCLEIC ACID EXCHANGE REACTIONS IN PNEUMOCOCCAL CAPSULAR TYPE TRANSFORMATION

A Preliminary Note

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The majority of experiments concerned with the transformation of pneumococci from one capsular type to another have included several steps. In the first of these reactions, an unencapsulated mutant is derived by selective cultural techniques from a type-specific, encapsulated strain of pneumococcus. The selected mutant, which fails to produce capsular polysaccharide of any type, is transformed then in the presence of the desoxyribonucleic acids of an encapsulated pneumococcus of heterologous or homologous type and acquires thereby the ability again to produce a capsule. In an experiment of this kind, the type of capsular polysaccharide produced by the transformed cells is determined by the capsular type of the pneumococci from which the transforming principle is obtained.

The possibility that pneumococci of one capsular type may be transformed directly to a second type without the intermediation of the unencapsulated variant of the strain to be transformed is an interesting one though few data pertaining to this subject are available. In 1931, before many details of the transformation reaction were known, Dawson and Warbasse (1) attempted capsular type transformation of inocula of encapsulated pneumococci. Using an encapsulated strain of pneumococcus type II grown in a medium containing type I anticapsular rabbit serum and a transforming vaccine of heat-killed type III pneumococci, they succeeded in recovering occasional colonies of pneumococcus type III from platings of the original culture. Although the development of competent, unencapsulated variants of the type II strain during the course of the experiment could not be excluded, the authors considered it possible that direct transformation from capsular type II to type III had occurred.

Two later observations by Taylor have provided information which seems pertinent to the problem investigated initially by Dawson and Warbasse. In the first of these observations (2), it was noted that the diffusely growing,

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diplococcal, unencapsulated variant of pneumococcus (R) and the autoagglutinable, chain-forming, unencapsulated variant (ER) could be transformed reciprocally. To explain this phenomenon, Taylor suggested that an exchange reaction might take place between desoxyribonucleic acids within the cell and those contained in the transforming principle in its environment. If the hypothesis is correct and representative of a general biological phenomenon, then it would be reasonable to anticipate that capsular transformation could take place in an analogous fashion. In a second series of observations concerned with the hereditary control of the quantitative aspects of capsular polysaccharide production in pneumococcus type III (3), Taylor has shown it possible for encapsulated pneumococci to participate in transformation reactions under conditions which preclude largely, if not entirely, the intermediation of unencapsulated variants. This result also supports indirectly the possibility that encapsulated pneumococci might be transformed directly to an heterologous capsular type.

Recently experiments describing "the induction of heritable new type in type-specific strains of *H. influenzae*" have been reported by Alexander and Leidy (4). These experiments were performed in the presence of anticapsular serum directed against the strain of *H. influenzae* to be transformed, a procedure which, in pneumococcus, creates selection pressure favoring the development of unencapsulated forms.

The experiments to be reported here describe capsular type transformation in pneumococcus carried out with inocula of fully encapsulated strains and under conditions not favoring the development of unencapsulated variants.

MATERIALS AND METHODS

1. *Preparation of Transforming Extracts, Anti-R Serum and Transformation Reactions in Vitro.*—The techniques employed were those described by MacLeod and Krauss (5).

2. *Crystalline Desoxyribonuclease.*—Five mgm. of crystalline bovine pancreatic desoxyribonuclease (Worthington Biochemical Sales Co. lot D333) were dissolved in 10 cc. of charcoal-absorbed broth containing 0.02M $MgSO_4$. The enzyme solution was sterilized by passage through a Swinny filter and stored at 4°C. The stock solution was diluted prior to use.

3. *Strains of Pneumococcus.*—II-D39S: A fully encapsulated strain of pneumococcus type II.

R36NC, R36NDH5: two unencapsulated variants of the type II strain, II-D39S.

I-DRM, III-A66, III-A66RM, VIII-B: fully encapsulated strains of pneumococcus types I, III, and VIII, respectively.

R36NC-TII, R36NC-TVIII: fully encapsulated strains of pneumococcus type II and type VIII, respectively, obtained by transforming strain R36NC with the transforming principles of strain II-D39S or strain VIII-B.

R36NDH5-TVIII: a fully encapsulated strain of pneumococcus type VIII obtained by transforming strain R36NDH5 with the transforming principle of pneumococcus VIII-B.

EXPERIMENTAL

To ensure insofar as possible the participation in the experiments of encapsulated pneumococci capable of being transformed, the two competent unencapsulated strains, R36NC and R36NDH5 were transformed to capsular types II or VIII. Inocula from single clones of the transformed encapsulated strains were seeded then into tubes containing 2 cc. of charcoal-absorbed broth, 5 per cent human pleural fluid agglutinating unencapsulated pneumococci, and transforming principle derived from an encapsulated pneumococcus of heterologous capsular type. In each experiment, control tubes lacking transforming principle were included. The cultures, which contained no anticapsular serum of any type at this time and therefore grew diffusely, were incubated for 24 hours at 37°C. After this initial incubation period, a loopful of each culture was streaked on a blood agar plate and quellung preparations were made. Examination of the plated subcultures revealed only the typically mucoid colonies of fully encapsulated pneumococci and the quellung preparations showed only

TABLE I

Biological Activity of Desoxyribonuclease on Pneumococcal Transforming Principles

STRAIN	TRANSFORMING PRINCIPLE	DESOXYRIBONUCLEASE	CAPSULAR TYPE
R36NC	I-DRM	+	−
		−	I
R36NC	II-D39S	+	−
		−	II
R36NC	III-A66	+	−
		−	III
R36NC	III-A66RM	+	−
		−	III

encapsulated pneumococci of the type used to inoculate the culture. After these controls had been made, 10γ of crystalline desoxyribonuclease in 0.2 cc. of charcoal-absorbed broth were introduced into each tube and the tubes were reincubated for one hour at 37°C. Simultaneuosly a test of enzymatic activity was carried out to ensure the ability of the desoxyribonuclease to inhibit the transformation of the unencapsulated pneumococcus, R36NC, in the presence of the transforming principle employed in the experimental tubes. As recorded in Table I, the inhibition of transforming activity in each instance was complete. After the incubation of the experimental tubes in the presence of desoxyribonuclease, 1.6 cc. of each culture was transferred to a second tube containing 4 cc. of 10 per cent anticapsular rabbit serum directed against the inoculated strain in neopeptone broth. The cultures were incubated overnight and the diffuse growth in the supernatant fluid was examined by plating and quellung techniques. In each instance, as shown in Table II, cells of the same capsular type as the cells from which the transforming principle in the experimental

tube had been obtained were demonstrated. Study of the plated subcultures in one instance revealed the presence of rare colonies of unencapsulated pneumococci in the agglutinated precipitate, but these forms were noted only after destruction of the transforming principle by desoxyribonuclease and incubation of the contents of the experimental tube in the presence of homologous anticapsular serum.

TABLE II

Pneumococcal Capsular Type Transformations Occurring After Inoculation of Encapsulated Strains

INOCULUM	TRANSFORMING PRINCIPLE	CAPSULAR TYPES RECOVERED
R36NC-TII	III-A66RM	II, III
R36NC-TVIII	III-A66	VIII, III
R36NDH5-TVIII	I-DRM	VIII, I
	II-D39S	VIII, II
	III-A66	VIII, III

DISCUSSION

The experiments reported here confirm the observation of Dawson and Warbasse (1) that encapsulated pneumococci may undergo capsular type transformation when grown in the presence of the transforming principle obtained from organisms of heterologous capsular type. Whether the reaction takes place as a result of the exchange of desoxyribonucleic acid within the cell for that introduced into the environment or through the intermediation of spontaneously arising, unencapsulated mutants during the growth of the cultures cannot be stated with certainty. The participation in reciprocal transformations of the diplococcal (R) and chain-forming (ER) unencapsulated variants of pneumococcus described by Taylor (2) has been considered explicable on the basis of nucleic acid exchange reactions and it has been shown also by Taylor (3) that encapsulated pneumococci may participate in transformation reactions. Both observations lend credence to the idea that transformation of capsular type in pneumococcus may take place through the medium of nucleic acid exchange reactions. On the other hand, no technique appears available at present whereby the possible appearance and subsequent transformation of unencapsulated mutant pneumococci during the course of the experiment can be excluded. Although the inclusion of antibody against unencapsulated pneumococci in the experimental system maintains selective pressure against unencapsulated forms (5), it cannot preclude their transient appearance and development. Until some more precise method, which of itself does not exert selective pressure, becomes available for the detection of minute numbers of unencapsulated mutants arising during the growth of encapsulated forms, the mechanism of capsular transformation under the conditions described must remain uncertain. The same arguments would seem to apply with equal force to the

question of capsular type transformation in *H. influenzae* as described by Alexander and Leidy (4).

SUMMARY

The phenomenon of pneumococcal capsular type transformation in experiments employing encapsulated pneumococci as the inoculum is described and discussed.

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MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

MONDAY, NOVEMBER 12, 1951

Dr. Grafflin: I would like to welcome you all to this meeting of the Johns Hopkins Medical Society. We have an interesting and a long meeting planned and I would like to start it with no further ado.

The first paper is by Drs. E. C. Andrus, A. L. Blalock and R. J. Bing. The presentation will be by Dr. Andrus.

THE SURGICAL TREATMENT OF MITRAL STENOSIS AND ITS PHYSIOLOGICAL CONSEQUENCES

A large series of patients with mitral stenosis has been studied in various clinics and laboratories in this hospital over the past two years. Of these, forty-two have undergone operation by Dr. Blalock.

Physiological studies by Dr. Bing with the technique of cardiac catheterization have demonstrated the fundamental circulatory disability characteristic of mitral stenosis. Whereas in the normal person pulmonary blood flow may be increased several fold upon demand without significant rise in pulmonary artery pressure, in the patient with mitral stenosis the pulmonary arterial pressure, already elevated at rest, rises considerably with exertion.

The indications for operation stem directly from the dynamic consequences of mitral stenosis. Dyspnea, often accompanied by cough, and transient pulmonary edema with exertion or excitement are evidently associated with a critical rise in the already elevated hydraulic pressure in the pulmonary capillaries. Hemoptysis from ruptured varices of the bronchial veins marks a sudden rise of pressure in collateral channels which provide a vent for blood from the turgid pulmonary venous system.

Certain features of the disease when present indicate that operation would be forbiddingly hazardous or unsuccessful. These are bacterial endocarditis, active rheumatic disease, obstructive disease of the aortic valve, intractable right heart failure, and significant mitral insufficiency.

Among the thirty-six patients, operated upon prior to October 1, 1951, there were two operative deaths and four operative failures. One of these four patients died fifteen months after operation. Of the remainder, the results in terms of functional improvement were excellent in seventeen, good in seven, and fair in six. The poor results followed in patients with long-standing congestion and evidently fixed pulmonic arterial resistance or in the presence of smoldering or recurrent rheumatic disease. The best were obtained in individuals under forty-five years of age whose disability, though evidently progressive, was minimal and of relatively short duration, and who displayed little or no cardiac enlargement.

By this experience certain conclusions regarding the vital pathology of mitral stenosis have received renewed emphasis. (1) This abnormality is often surprisingly well tolerated for a long period; then disability may set in and progress rapidly. (2) Beginning as a disease of a heart valve, it becomes a disease of the lung and of the pulmonary circulation which at length overwhelms the strength of the right ventricle. (3) From the standpoint of probable improvement after surgery, the key to the problem evidently lies in the lungs: the effects of long-standing pulmonary congestion and the presence or the development of concomitant inflammatory reactions involving the pulmonary vasculature and alveolar walls. (4) The

tests applied to determine the presence of "active" rheumatic disease in the heart have not proved adequate in all cases.

To bring help to patients in the more advanced stages of this disease, new knowledge is required in at least three directions: (1) more adequate description in functional terms of the abnormalities in the lungs which accompany mitral stenosis, and the effects of the relief of mitral obstruction upon these abnormalities, (2) a form of therapy which may reverse the pulmonary changes, and (3) means more accurately to detect and more effectively to treat rheumatic disease of smoldering degree or recurrent nature.

Dr. Grafflin: Dr. Blalock will speak to us on operative procedures directed to the relief of these cases. He has asked me to say that Miss Berger will introduce the subject with a brief discussion of preoperative and postoperative care.

Miss Berger: These patients have been evaluated by Dr. Andrus and his associates and come to surgery in the optimum condition. Adequate preoperative sedation and medication is of the utmost importance. A tense, apprehensive patient may develop pulmonary edema before anesthesia is administered. Pentobarbital 0.1 to 0.2 gram is administered the night before operation and repeated, if necessary on the next morning. Morphine 12 to 16 mgm. with atropine 0.6 mgm. is administered one and a half hours before anesthesia. At the discretion of the cardiologist atropine has been omitted for the occasional patient with excessive tachycardia. If the patient is orthopneic he is anesthetized in the sitting position.

We feel inhalation anesthesia is to be avoided for induction because pulmonary edema is frequently present. The edema interferes with diffusion of the anesthetic gases and the induction phase is protracted. This tends to increase the apprehension and augments the pulmonary edema. A vicious cycle occurs. Our first patient in this series was unwisely anesthetized with cyclopropane and oxygen. After a prolonged and stormy induction period massive pulmonary edema developed.

Sodium Pentothal 2.5% with 12 to 15 mgm. of d-tubo-curarine to facilitate intubation is now routinely employed for induction. Following intubation the anesthesia is maintained with ether and oxygen by the to and fro absorption technique. An occasional patient has been maintained on Pentothal and oxygen throughout surgery but our preference is for ether and oxygen. The choice of agents or techniques for maintenance of anesthesia probably should be governed by the usual practice of the individual anesthetist.

Early in the series an attempt was made to off-set hypotension by an intramuscular injection of methoxamine hydrochloride (Vasoxyl) at the time the chest was opened. The results were not satisfactory and we have discontinued the practice. Intravenous procaine or procaine amide (Pronestyl) was administered in the early cases in the hope of decreasing arrhythmias. We did not feel these substances were of material benefit as a routine practice and have discarded their use. We feel the emphasis should be placed on adequate ventilation with high oxygen mixtures rather than on the employment of a variety of drugs.

Because of the marked diminution in the filling of the left ventricle during commissurotomy, the radial pulse may become barely perceptible and the blood pressure not obtainable. This is of no great concern if the heart action is constantly visualized and noted to be effectual. The pulse and blood pressure promptly return toward the normal with removal of the finger or instrument from the mitral opening. Should hypotension develop before the chest is opened a small dose of a vasopressor drug may be administered.

There have been no operative deaths in the series. Two patients died in the postoperative period.

Dr. Blalock: This is a good opportunity to say how extremely important Miss Berger has been in the care of these patients, and I am sure Dr. Andrus and others will agree.

Dr. Blalock considered the efforts which were made to treat mitral stenosis by surgical means in the 1920's and contrasted the results then with those that have been obtained in the past several years. It is generally agreed, at present, that the approach should be through the left auricular appendage and that the two commissures should be torn with the index finger or cut with an especially devised knife. The great majority of the patients have been treated by finger fracture or tear. The operative mortality has been low and the other results are quite encouraging. Mitral commissurotomy seems to be a sound procedure.

Dr. Grafflin: I should like to call on Dr. Cooley, of the Department of Radiology, to discuss some of the radiological aspects of this work.

Dr. Cooley: Conventional roentgenographic studies have not contributed greatly to an understanding of the physiological problems associated with the surgical treatment of mitral stenosis. There has been a rough correlation in several of the operated cases between the size of the pulmonary artery as determined by roentgenography and the pulmonary artery pressure as determined by cardiac catheterization. However, the demonstration of an enlarged pulmonary artery does little towards clarifying the dynamics of the pulmonary circulation since such an enlargement may be associated with either an increased pressure such as occurs in mitral stenosis or an increased volume blood flow such as is seen in interatrial septal defect or a combination of these two factors. No precise analysis of the dynamics associated with pulmonary artery enlargment is thus permitted by routine roentgenographic studies.

The presence of mitral stenosis can apparently be determined in the majority of cases from the history and physical examination. In the selection of patients for operation, negative radiographic findings have usually been of more value than positive ones. The absence of enlargement of the left ventricle or of marked enlargement of the left atrium or the absence of diffuse changes in the pulmonary parenchyma have been more significant than the demonstration of slight enlargement of the left atrium or pulmonary artery. Calcium deposited in the mitral valve is almost pathognomonic of mitral valvular disease with stenosis predominating. Yet in almost every instance in which calcium was found the diagnosis of mitral stenosis had been strongly suspected from clinical findings.

Radiographic findings have frequently been minimal. In one instance a patient had had symptoms of mitral stenosis for three years and there were definite physical signs yet there were no detectable changes in the cardiac contour. In 13 patients in the present series, the presence of left atrial enlargement was open to question. It is logical to assume that typical murmurs appear earlier than any changes in the external contour of the heart and that definite clinical mitral stenosis may be present without any demonstrable roentgenographic changes.

A statistical analysis of the pertinent radiographic findings in the first 40 patients in this series of operated cases has been made. These figures do not warrant any definite conclusions but they are of some general interest since they may convey some idea of the type of patient chosen for operation and also something of the immediate results.

These figures were presented by means of lantern slides.

Films of the several of the operated patients in which there had been a follow-up of 6 months or longer were presented.

Dr. Grafflin: I would like to call on Dr. Sabiston of the Department of Surgery.

Dr. Sabiston: A histological study has been made of the left auricular appendage removed at the time of operation. The findings were interesting in that all showed evidence of pathological change, that is, none were normal. Perivascular scarring, hypertrophy of muscle fibers, round cell infiltration, fresh and organized thrombi, and fibrous thickening of the epicardium and endocardium were observed. Lesions definitely identifiable as Aschoff bodies were encountered in approximately two-thirds of the cases. It appears on the basis of the early follow-up that those patients without Aschoff lesions have a more favorable postoperative course.

Dr. Grafflin: Is there any further discussion of this interesting work? Dr. Riley?

Dr. Riley: The correlation between the pathological changes in the small pulmonary arteries and the increased resistance to flow as estimated from the pressure changes is very nice. We hope that the correlation between pathological changes in the alveolar walls and impairment of alveolar-capillary diffusion may also eventually be expanded a little.

Then there is the interesting question of the significance of the enlarged bronchial veins which may occur in mitral stenosis. If, as seems probable, these veins carry a larger than normal amount of blood, where does the additional blood come from? The pressure relationships are such as to suggest that the normal anastomosis between the pulmonary capillaries and the bronchial veins may be increased, and that the enlarged bronchial veins may be carrying a significant quantity of blood which has come from the pulmonary capillaries. In this case the bronchial veins would tend to decompress the pulmonary capillaries. The blood drained off in this way would pass back in systemic veins to the right side of the heart without passing through the left heart, and the output of the right heart would accordingly be greater than that of the left.

This is a possible aspect of mitral stenosis that is seldom discussed and that is difficult on technical grounds to evaluate.

Dr. Grafflin: Is there any further discussion? If not, the next paper is by Drs. Janet Jordan and George S. Mirick, and will be given by Dr. Mirick.

HEPATITIS IN MICE, OF PRESUMED VIRAL ORIGIN

The data presented have been published in an article appearing in the Bulletin of the Johns Hopkins Hospital, *89*, 326–331, (1951).

Dr. Grafflin: First, I should like to call on Dr. Janet Jordan, co-author of this presentation.

Dr. Jordan: I would like to stress again something that Dr. Mirick has already mentioned. (1) The fact that different lots of virus have been unpredictable with regard to infectivity in mice; (2) it was impossible to obtain titers beyond 10^{-2} in this disease; (3) the mice don't appear to be very sick. They become very fat and then within two or three weeks there is spontaneous diuresis with recovery.

Dr. Grafflin: Dr. Harvey.

Dr. Harvey: I think most of us realize the tremendous importance of the problem of hepatitis. Heretofore it has only been approachable from the investigative angle by human volunteer experiments which are extremely difficult to set up and somewhat hazardous.

Dr. Mirick and Dr. Jordan are to be congratulated for the perseverance they have shown in the face of many negative trials by other workers. I believe, personally, that they have discovered something that is going to be of tremendous importance and if it can be proven that this mouse infection is due to the human virus, it will undoubtedly open the way to great advances in this field.

Dr. Grafflin: Dr. Rich.

Dr. Rich: This is very interesting and important work. I think that those of us who have listened to it have to share Dr. Mirick's wonder whether the agent might be a spontaneous mouse virus. We very greatly hope that it is not. I would like to ask Dr. Mirick whether he has been able to produce ascites by intracerebral inoculation.

Dr. Mirick: I would like Dr. Jordan to answer that.

Dr. Jordan: One or two of the mice inoculated intracerebrally had a small amount of ascites. Those inoculated intravenously did develop ascites. We never observed as much ascites in the mice inoculated intracerebrally as in those inoculated intraperitoneally.

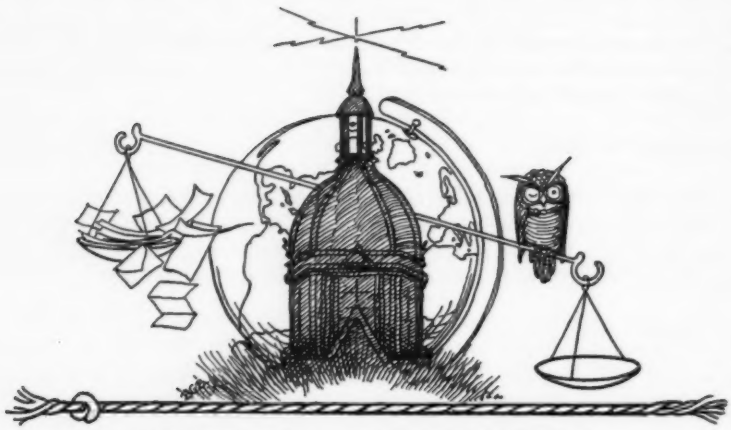
Dr. Rich: I wonder whether the local irritation from the intraperitoneal injection was responsible for the ascites, because infectious hepatitis itself is not characterized by ascites unless the patient survives a great amount of liver destruction. In these mice the amount of liver cell damage was minimal.

Dr. Mirick: I found it hard to understand. We wonder too and anatomically don't see any reason why they should have acites. It seems to be correlated with the degree of infiltration around the vessels and I imagine they could be completely blocked off in places and that might result from increased portal pressure. The fluid seems to contain too large an amount of protein for that. The kinds of cells and the low numbers of cells seem to argue against it being an inflammatory reaction, as does the appearance of the peritoneum, which is shining and benign looking in all cases.

Dr. Blalock: Did the mice develop increased portal pressure or esophageal varices?

Dr. Mirick: I can't answer that. We haven't measured portal pressure and haven't any information on that.

Dr. Grafflin: Is there any further discussion? If not, I would like to express our thanks to the speakers for an extremely interesting meeting which now stands adjourned.



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Guy's Hospital, *1725–1948.* Edited by HUJOHN A. RIPMAN. 176 pp., 18s. 6d. *Clarke and Sherwell, Northampton, England.*

This volume contains a short history of one of the world's most famous hospitals. The work is divided into sections, the first of which describes the origins of the founder, Thomas Guy, and the circumstances under which the hospital was founded. The physical development and expansion are fittingly outlined in connection with brief sketches of the outstanding personalities of the staff, those who contributed by their administrative zeal and donations, as well as the extraordinary succession of superb clinicians who have graced the hospital throughout its life.

Other chapters are devoted to the founding of the medical, dental and nursing schools, again with special emphasis on the personalities whose contributions made possible the rapid development and fame of these schools.

The final chapter gives a picture of the entire physical plant as it now stands, together with a precise description of the administrative organization.

The book is beautifully illustrated and well printed. It makes fascinating reading for those interested in medical history and the evolution of our great modern hospitals. To the host of friends of Guy's who are scattered all over the world, this volume will prove a most welcome contribution.

J. E. H.

Tumors of the Head and Neck. By GRANT E. WARD and JAMES W. HENDRICK. 832 pp., $15.00. *The Williams & Wilkins Co., Baltimore, Md.*

Dr. Ward has long written authoritatively on tumor surgery, and the present volume stems from his extensive experience at the tumor clinics of The University of Maryland, and The Johns Hopkins Hospitals.

The large (832 pages) volume is in most respects an excellent example of modern text book publishing. It is profusely illustrated with clinical photographs, photomicrographs and drawings in general of high quality and clarity. There is a generous use of color. The color illustrations are well selected and appear to have been chosen for the additional help derived from color reproduction, unlike some recent works—rich in color reproductions seemingly chosen more for their chromatic appeal than for their value in assisting the text. Several special sections,—tumors of the eye and adnexa, tumors of the skull, plastic rehabilitation, tumors of the para-nasal sinuses and the chapter on embryology of the head and neck and others are the work of contributors. The currently popular double column format is used.

The volume is informative, authoritative, and the text covers an extraordinarily wide and complete range of conditions. The text and illustrations are particularly good and valuable in describing operative techniques and the most is made of the illustrations by providing them with detailed legends. Perhaps the chief complaint which can be made is against a certain awkwardness and looseness in the language reminiscent of a casual conversational style, at times replaced by a labored prosiness that is scarcely less distracting and is not helped by an occasional touch of lightness or the introduction of a faintly relevant proverb. The legends are frequently awkwardly written, despite their valuable content, and one could wish there were some uniformity of style in them. Of a piece with this is the careless orthography—"gallop poll," "accidently," "pre pubital."

There is an excellent detailed section on neck dissections with a good deal of emphasis on the composite operation for radical neck dissection with removal of cancer of the mouth. The operation, devised by Ward and Edgerton, brings the radical operative treatment of lesions of the mouth and tonsil, to what would appear to be the ultimate in excision therapy. Antibiotics, modern plastic techniques, and the feasibility under present conditions of long and tedious and detailed procedures have encouraged the reinvestigation of the value of radical operations abandoned almost 50 years ago as too mutilating and dangerous. It is apparent that the initial hazard has been greatly reduced and intelligent planning minimizes subsequent deformity and disability. It remains to be seen what the ultimate salvage will be.

Throughout the book, multiple therapeutic possibilities are described and as much space is given to methods of others as to those of the authors. Dr. Ward has had a long experience with the use of radiant energy in the treatment of tumors so that the discussions of the varying applicability to given lesions of irradiation and operation are given special weight and value and the suggestions for the use of radiant energy are as detailed and precise as the descriptions of the operative methods.

M. R.

Paracelsus. Magic into Science. By HENRY M. PACHTER. 360 pp., $4.00. *Henry Schuman, Inc., New York.*

Within the framework of a biography of the famous Swiss physician Theophrastus of Hohenheim, called Paracelsus (1493–1541), the author tries to depict the development of this controversial figure and to evaluate his significance in the history of science and philosophy. Disgusted with the traditional learning, Paracelsus, in Pachter's opinion, turned to surgery without acquiring an M.D. degree, and then traversed the world in quest of medical knowledge. Practicing, writing, and teaching (for a short period in Basel) he evolved a naturalistic theory which culminated in the concept of nature as a unity ruled by laws which include man, yet allow him biological as well as spiritual individuality. During this period, Paracelsus was the "magus," foreshadowing the modern scientist in

his quest for useful knowledge. Then, in 1532, a religious conversion made him abandon his faith in "science," and his last years (which the author treats very briefly) were marked by a return to medicine in a chastised mood of more conventional religiosity.

The author tries to view Paracelsus objectively without hate or adulation. Many of his comments are stimulating, e.g. his characterization of magic as "science in the Utopian stage" (p. 328) or his contention that Paracelsus was influenced by the philosophy of Duns Scotus. The book will be of value chiefly to those whose familiarity with Paracelsus will enable them to read it critically. Others, however, and this probably means just those for whom it is primarily intended, have to be cautioned. We simply do not know enough about Paracelsus' life before 1525; his autobiographical remarks are too vague and the chronology of his early works too uncertain to allow us an insight into his development. Much of the biography (e.g. the schedule of his travels) rests on very vague evidence and the author, unfortunately is not circumspect in the use of his material. Sometimes the references cannot be found where quoted (e.g. p. 49, note 2, p. 68, note 6, p. 220, note 1), sometimes they do not bear out the author's claims (e.g. p. 104, note 1, has no reference to Salzburg), and sometimes the author's contention is as dogmatic as that of the Paracelsus worshippers he attacks. Thus he denies that Paracelsus had an M.D. degree. He may be right; the main evidence for this degree rests on Paracelsus himself. When the latter had to appear as a witness in court (in Basel, not Strasbourg as Pachter, p. 308, note 15, has it), he gave his testimony "by the oath which he had sworn to the renowned university at Ferrara on his admission to the doctorate."[1] But if Paracelsus lied on such an occasion, it is hard to say where he should be believed.[2] The shortcomings of the biographical parts of the book are repeated in the interpretative passages. The author is too easily satisfied with sketching an interesting idea with insufficient proof. For instance, the exaggerated contrast between Paracelsus' earlier pantheistic leanings and later theism (see p. 256 f.) seems to rest on a doubtful work (p. 257, note 13) and doubtful interpretation (the quotation of note 15 refers to the disposition of a book, the "Philosophia magna," not to his life). In style and thought the book often resembles a journalistic synthesis rather than a patient historical interpretation.

O. T.

Diabetes Insipidus. By HARRY BLOTNER. Edited by HENRY A. CHRISTIAN. Reprinted from Oxford Loose-Leaf Medicine. 200 pp., $4.50. *Oxford University Press, New York.*

This monograph, reprinted from Oxford Loose-Leaf Medicine, is written by one who has had a broad experience with the disease. The literature is fairly completely reviewed up to 1948; the bibliography alone consumes 33 pages.

The anatomy, physiology, pathology and treatment are thoroughly covered. Dr. Blotner presents his theories on the pathology and pathological physiology of the disorder, and many will take issue with his concepts of the importance of fat and cholesterol metabolism in the pathogenesis of diabetes insipidus.

Particularly valuable is the discussion of the various posterior pituitary preparations and their relative cost. It is clearly shown that intranasal posterior pituitary powder, if tolerated by the patient, is the treatment of choice. An error appears in a table on p. 137 where it is stated that obstetrical and surgical pituitrin are of the same strength, while in the text it is correctly stated that surgical pituitrin is twice the strength of obstetrical.

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[1] See Albrecht Burckhardt in *Correspondenz-Blatt für Schweizer Aerzte*, **44**: 884, 1914.

[2] Pachter's admission that Paracelsus might have been a mere doctor of surgery seems an *ad hoc* hypothesis to save Paracelsus from the accusation of perjury.

There is no mention of the hypertonic saline infusion test in the section on diagnosis. Some physicians have found this test of value in differentiating psychogenic polydipsia and polyuria from diabetes insipidus; the author's comments on the value of the test would have been of great interest.

In summary, the book is valuable as a reference but is not recommended for the practising physician.

H. F. K., Jr.

The Normal Cerebral Angiogram. By ARTHUR ECKER. 190 pp., $6.50. *Charles C. Thomas, Springfield, Illinois.*

This book represents a timely publication in the relatively new field of cerebral angiography. Because it is a new field this work can in no way be considered an enduring classic. It should be regarded more as a stimulus for the study of intracranial lesions by a method which shows increasing popularity.

The book consists of 216 pages with 147 illustrations. Part I is concerned with Dr. Ecker's cerebral angiographic technique. Naturally each hospital with any experience in cerebral angiography has developed its own technique and there is much variation in the technical operations as well as the equipment. Dr. Ecker's elaboration of his technique may be of considerable aid as an example of a successful method which can be studied by physicians interested in initiating cerebral angiography.

It would be desirable if the author made clear exactly how many cerebral angiograms he has performed as the basis for his experience with the normal angiogram. It would also be interesting to have more statistics of the variations from the normal together with the actual measurements. For example the measurements in millimeters of deviation of the anterior cerebral artery from the midline would be useful information.

Of particular value is the author's discussion of the relationship of the pressures in the carotid and intracerebral vessels to the exact pattern of the flow of diodrast.

This book is a laudable and timely instruction which lays the anatomical foundation for the interpretation of cerebral angiograms.

D. M. G.

Pharmacologic Principles of Medical Practice, 2nd Edition. By JOHN C. KRANTZ JR. and C. JELLEFF CARR. 1116 pp., $10.00. *The Williams & Wilkins Co., Baltimore, Md.*

Nowhere in medical education is there a greater need for a good text than in the field of pharmacology. The second edition of Krantz and Carr has in no way lessened this need. The weaknesses of this text are apparent in both the pharmacological and clinical discussions. On the pharmacological side there is an abundance of pharmaceutical and historical material together with numerous items from the current literature. In between there is a dearth of primary data on the disposition of drugs in the body. Concerning clinical matters, the book is equally feeble. The authors give the impression that they are unable to define precisely the sphere of usefulness of any given drug, and instead give it a vague and general blessing. For example, in the discussion of emetine, the reader cannot discover whether it is meant for use in intestinal or hepatic amebiasis. Again, in regard to quinidine one gains the conviction that it should be used whenever any cardiac arrythmia is present. There are in addition a number of errors of fact and omission. The bibliographies are inadequate and do not include some of the key references mentioned in the text.

This book, in brief, fails of its purpose because in its pharmacology it is empty where it should be full and in its therapeutics it is vague where it should be specific. It cannot be recommended for medical students or physicians.

C. G. Z.

BOOKS RECEIVED FOR REVIEW

Advances in Tuberculosis Research, vol. 5. Edited by H. BIRKHÄUSER and H. BLOCH. 308 pp., $10.50. *S. Karger, Ltd., Basle, Switzerland.*

The Biochemistry of Fertilization and the Gametes (Biochemical Society Symposium No. 7). 66 pp., 9s. 6d. net. *Cambridge University Press, New York.*

Biological Antagonism. The Theory of Biological Relativity. By GUSTAV J. MARTIN. 516 pp., $8.50. *The Blakiston Co., Philadelphia, Pa.*

Clinical Methods, 12th edition. By SIR ROBERT HUTCHISON and DONALD HUNTER. 484 pp., $5.00. *J. B. Lippincott Co., Philadelphia, Pa.*

Comparative Physiology of the Thyroid and Parathyroid Glands. By WALTER FLEISCHMANN. 78 pp., $2.25. *Charles C. Thomas, Springfield, Illinois.*

Conteben Bei Lungentuberkulose. By H. KLEESATTEL and W. GÜRICH. 154 pp., Kartoniert DM 22.50. *Georg Thieme Verlag, Stuttgart, Germany.*

Handbook of Diseases of the Blood. By A. PINEY. 213 pp., $5.00. *J. B. Lippincott Co., Philadelphia, Pa.*

Histologie und Mikroskopische Anatomie des Menschen, vol. 1. By WOLFGANG BARGMANN. 210 pp., geb. DM 19.60. *Georg Thieme Verlag, Stuttgart, Germany.*

Histologie und Mikroskopische, vol. 2. By WOLFGANG BARGMANN. 563 pp., Ganzleinen DM 45. *Georg Thieme Verlag, Stuttgart, Germany.*

Kymographische Röntgendiagnostik. By PLEIKART STUMPF. 120 pp., Kartoniert DM 25.50. *Georg Thieme Verlag, Stuttgart, Germany.*

Plastic Surgery of the Nose. By JAMES BARRETT BROWN and FRANK MCDOWELL. 427 pp., $15.00. *The C. V. Mosby Co., St. Louis, Missouri.*

Practical Therapeutics, 2nd edition. By MARTIN EMIL REHFUSS and ALISON HOWE PRICE 938 pp., $15.00. *The Williams & Wilkins Co., Baltimore, Md.*

The Specialties in General Practice. Edited by RUSSELL L. CECIL. 818 pp., $14.50. *W. B. Saunders Co., Philadelphia, Pa.*

Studies in Medicine. A Volume of Papers in Honor of Robert Wood Keeton. By Various Authors. 396 pp., $8.50. *Charles C. Thomas, Springfield, Illinois.*

Textbook of Refraction. By EDWIN FORBES TAIT. 418 pp., $8.00. *W. B. Saunders Co., Philadelphia, Pa.*

Topographische Ausdeutung der Bronchien im Röntgenbild. By CLAUS ESSER. 152 pp., Kartoniert DM 33. *Georg Thieme Verlag, Stuttgart, Germany.*

Untoward Reactions of Cortisone and ACTH. By VINCENT J. DERBES and THOMAS E. WEISS. (A Monograph in American Lectures in Internal Medicine. Edited by ROSCOE L. PULLEN.) 77 pp., $2.25. *Charles C. Thomas, Springfield, Illinois.*

The Will to Live. By ARNOLD A. HUTSCHNECKER. 278 pp., $3.50. *Thomas Y. Crowell Co., New York.*